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DISSOLVED OXYGEN DEPLETION DUE TO NATURAL ORGANIC RUNOFF IN THE RED DEER RIVER SYSTEM

by

© STANLEY E. PENTTINEN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled DISSOLVED OXYGEN DEPLETION DUE TO NATURAL ORGANIC RUNOFF IN THE RED DEER RIVER SYSTEM submitted by STANLEY E. PENTTINEN in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ENVIRONMENTAL SCIENCE.

ABSTRACT

The Red Deer River suffers chronic low dissolved oxygen levels during periods of winter ice cover. This problem has been evident for several years. However, previous studies have not been able to account for this dissolved oxygen depletion in terms of known man-made inputs or existing readily biodegradable natural organics. An experimental program was designed to assess the possible nature and the approximate bounds of oxygen demand due to natural organic runoff in the Red Deer River system. Samples from a site on the Blindman River, a tributary of the Red Deer River, were used in this investigation. The water contained no significant quantities of man-made input, it was high in organic carbon and it was highly coloured due to muskeg leaching. The study involved the use of an electrolytic respirometer on stream water samples which were concentrated by vacuum evaporation.

Carbon and nitrogen budgets in conjunction with the measured oxygen demand at 20°C and at temperatures approaching winter, under ice, conditions, indicate that significant biochemical oxygen demand is originating with natural organic runoff and can be observed at low temperatures. Also, the role of nitrification appears to be quite significant in the measured oxygen uptake. However, due to the presence of aquatic humic substances, which are capable of scavenging various nitrogenous compounds, an accurate assessment of the degree of nitrification was not

obtained. An application of the results of this investigation in conjunction with an evaluation of the relative flow data of the Blindman River and similar tributaries in this area, provide an estimate of the contribution of natural organic runoff to the observed oxygen demand in the Red Deer River.

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I. INTRODUCTION

Oxygen Regime in Rivers

Within a river system the degree of "self-purification" and the presence of aquatic life is highly dependent upon the dissolved oxygen (DO) concentration. Dissolved oxygen is therefore regarded as one of the most important parameters in assessing river water quality. The dissolved oxygen level depends on the relationship of the rates of oxygen supply, through atmospheric reaeration and photosynthesis and the oxygen demand, primarily through respiration.

In open river waters the dissolved oxygen concentration is readily maintained at acceptable levels, assuming reasonable control over the degree of man-made organic loading. However, in northern climates, the period of winter ice cover greatly, if not entirely reduces the opportunity for reaeration and photosynthesis. The problem is compounded during this period due to reduced flow rates. Therefore, if the oxygen demand rates are sufficiently high and the river is long, the dissolved oxygen concentration will eventually fall to undesirable levels. Such instances have been observed in pristine river stretches and in stretches affected by municipal and industrial wastes (Mosevich, 1947; Drachev, 1962; and Gordon, 1970).

Dissolved oxygen under ice cover has been a subject of concern to a number of investigators (Drachev, 1962; Cameron, 1967; Gordon, 1970; Landine, 1970; Bouthillier and Simpson, 1972; Chapman, 1972; and Baker *et al*, 1975 and

1977). This continuous process of DO depletion throughout most of the period of ice cover is yet not entirely understood. The need for a greater understanding is required to obtain better methods of predicting DO levels under ice conditions due to increased development in northern regions and a greater concern for the state of our environment.

The Red Deer River System

The Red Deer River rises in the Rocky Mountains in Alberta and drains into the South Saskatchewan River near the Alberta-Saskatchewan border. The channel bed is primarily gravel except in the lower reaches where it is sand. In the Red Deer River basin (Figure I-1), the water is relatively clear and the river maintains its mountain stream characteristics as far as Red Deer. The winter flow rates have been less than 3 m^3/s. This portion of the river and its tributaries flow through large portions of muskeg and cultivated areas. These tributaries can account for as much as 30% of the winter flow in the Red Deer River.

The Red Deer River in central Alberta has been intensively studied for several years to assess the ability of the river to meet the demands of speculated municipal and industrial growth in and around the city of Red Deer. The primary problem is the chronically low dissolved oxygen levels (less than 5 mg/l) which occur during winter ice cover.

The problem of low dissolved oxygen concentrations

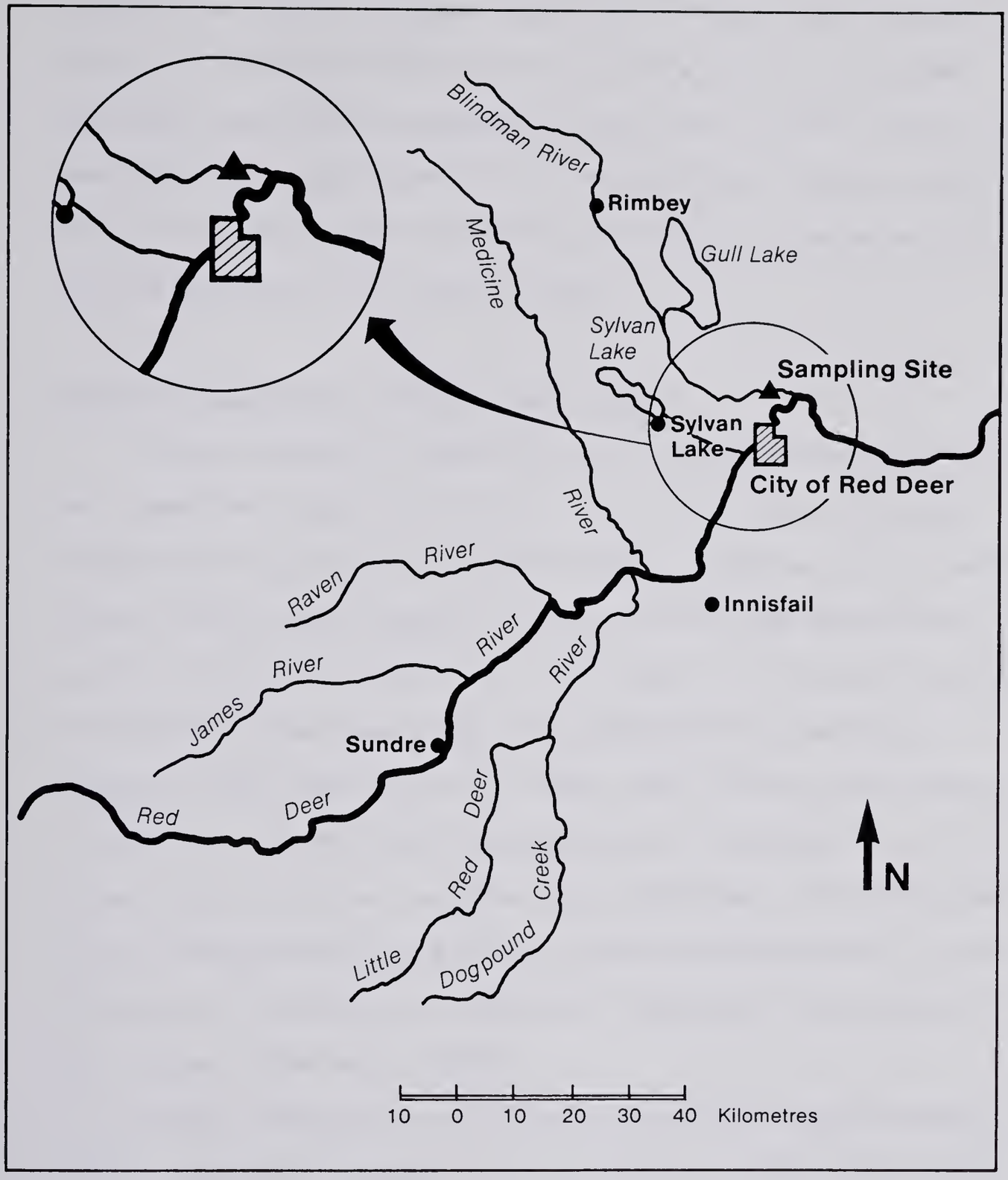


FIGURE I-1. Red Deer River Basin in Central Alberta.

below the city of Red Deer during winter ice cover has been evident for several years. The most obvious contributing factor to produce this situation is the amount of oxygen demanding materials released to the river by the city of Red Deer and other municipalities. Another contributing factor may be the amount of naturally occurring oxygen demanding materials within the river system.

Earlier Studies On The Red Deer River

In the winter of 1969-70 Alberta Environment found that the dissolved oxygen reached a low of 0.7 mg/l, 185 km downstream from the city of Red Deer, which is the largest source of man-made organic loading in the Red Deer River system. Studies conducted in the winter of 1973 by Alberta Environment (Red Deer River Flow Regulation Planning Studies, 1975) show that, at most, only 32% of the oxygen consumed in the Red Deer River from the confluence of the Raven River (80.5 km upstream from Red Deer) to Drumheller (48 km downstream from Red Deer) can be attributed to the biological stabilization of waste discharged from the Red Deer Sewage Treatment Plant.

During the winter of 1970-71 Bouthillier and Simpson (1972) reported findings of a traverse of 130 km of river, including 48 km upstream of the city of Red Deer. The upstream flow, which experiences no man-made organic loading, had chemical oxygen demand (COD) values as high as 16 mg/l and on the average, had a total organic carbon (TOC)

value of 5 mg/l, and a biochemical oxygen demand ($BOD_{5,20}$) of 1 mg/l. At the city of Red Deer the DO level was 8 mg/l (this is unusually low since the saturation value for DO at 0°C and at an elevation of 670.6 m is 13.5 mg/l) which indicated a 1.3 mg/l depletion of dissolved oxygen in the 48 km stretch immediately upstream of Red Deer. This natural oxygen demand imposed greater constraints upon the Red Deer Sewage Treatment Plant to maintain desirable levels of dissolved oxygen in the Red Deer River.

Figures I-2 and I-3 show data obtained by Bouthillier and Simpson (1972) in the winter of 1970-71 on river flow, dissolved oxygen and biochemical oxygen demand.

Baker et al. (1975 and 1977) performed intensive studies on the organic water quality and microbial ecology of the Red Deer River basin. These studies showed that organic matter is relatively abundant in these waters with seasonal variations (TOC concentrations of 24 mg/l were common in some tributaries). The organic matter inputs to the stream are primarily due to leachate from vegetation litter and muskeg soils. Removal pathways of organic matter from the stream include deposition (through processes of precipitation, adsorption, absorption, etc.), biological and chemical degradation as well as stream outwash. The tributaries examined generally had higher levels of organic matter, were more highly coloured and during winter ice cover, had much lower DO levels than the Red Deer River.

Baker et al. (1975) analysed for specific organic

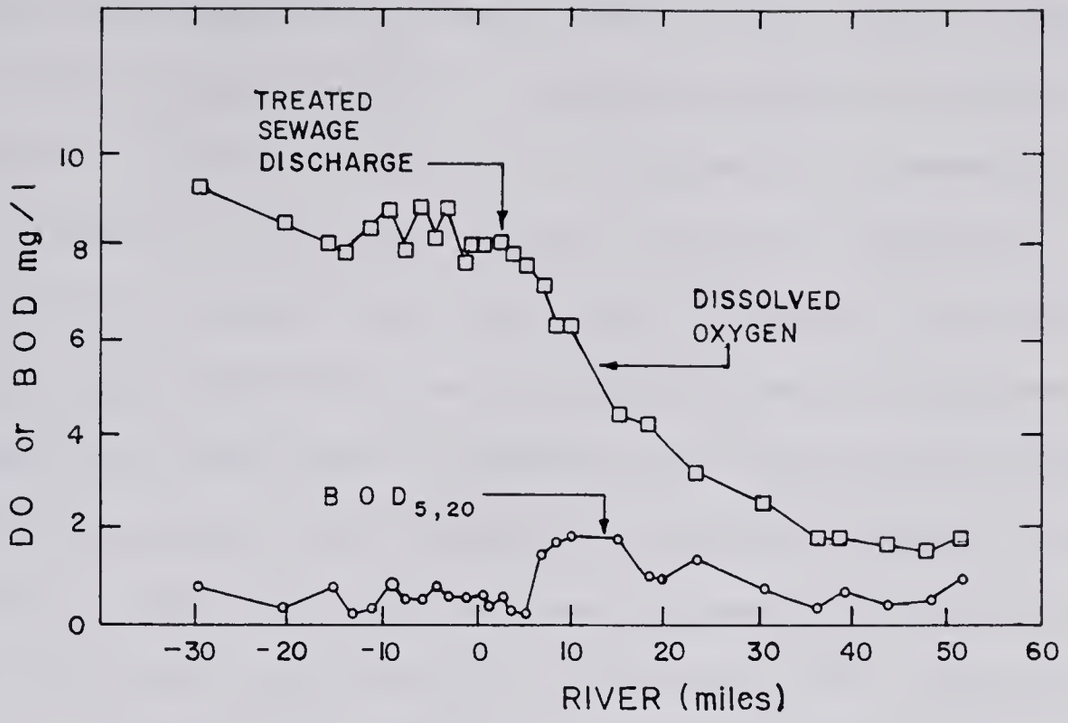


FIGURE I-2. Dissolved oxygen and biochemical oxygen demand profiles of the Red Deer River, Jan. 20, 1971 (from Bouthillier and Simpson, 1971).

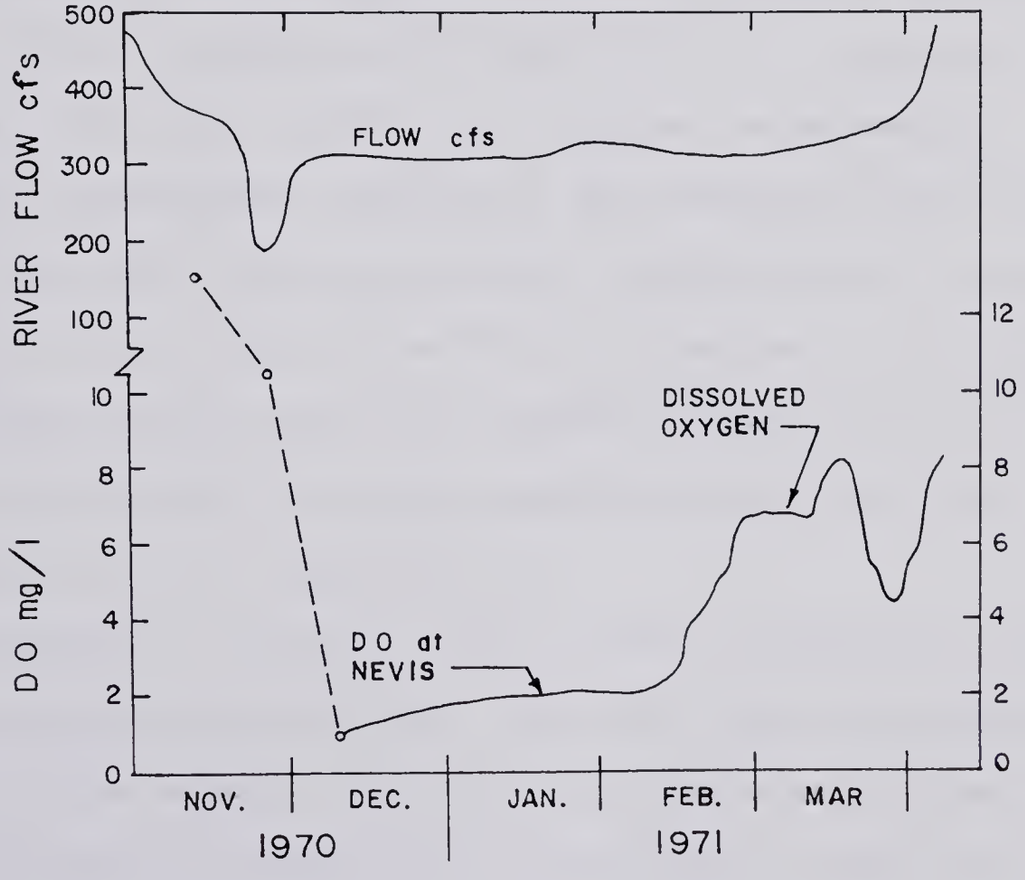


FIGURE I-3. Dissolved oxygen levels in the Red Deer River at Nevis (84 km downstream from the City of Red Deer) and the corresponding river flow rates (adapted from Bouthillier and Simpson, 1971).

compounds and compound classes (Table I-1). Fatty acids, amino acids, hydrocarbons and phenolics were not found in sufficient quantity to explain the observed biochemical oxygen demand on the basis of any plausible oxidation stoichiometry. On the other hand the levels of total organic carbon, humic and fulvic acids and tannins and lignins were of sufficient magnitude to suggest that biological oxidation of some portion of the organic matter represented by these parameters could explain observed biochemical oxygen demand. Humic and fulvic acids were more abundant than tannins and lignins, probably due to the fact that there is a greater absolute quantity of humic substances on the land surface.

Microbial investigations reported by Baker et al. (1977) indicate that greater than 99% of the bacteria in the Red Deer River are sessile and attached to submerged surfaces and sediment particles. The biological degradation of the dissolved organics has been attributed to these organisms. This supports earlier speculation (Bouthillier and Simpson, 1972) that the observed oxygen depletion was caused by attached microbial populations on the river bed exerting a benthic oxygen demand.

In order to meintain a high level of water quality it is necessary that sufficient dissolved oxygen is available for the large sessile bacterial population to metabolize the dissolved organic matter. During periods of open water the DO level is sufficient to satisfy the needs of the sessile population. However, during ice cover, the sessile

TABLE I-1. Data Summary of Some Compound Classes Found in the Red Deer River Basin (Baker et al, 1975).

Compound Class (Date of Analysis)	Summary of Analysis
Fatty Acids (May and June, 1975)	-total fatty acids = 6 to 28 µg/l (C_{18}-C_{27}, saturated and unsaturated) -free fatty acids and fatty acid esters contributed equally to total -unsaturated fatty acids were about 1% of total
Amino Acids (June'74 - June'75)	-free amino acids = 0.8 to 23.1 µg/l combined amino acids = 1.9 to 142 µg/l -free amino acids accounted for only about 15% of the total amino acids -highest values occurred in the Spring
Hydrocarbons (N.A.)	-total normal alkanes = 0.12 µg/l (Red Deer River) and 0.27 µg/l (Medicine River) (C_{12}-C_{31}) -isoprenoid, cyclic and branched hydrocarbons were less abundant
Phenolics (June - Sept., 1975)	-2 to 17 µg/l (most were above the standard level for phenolics, 5 µg/l) -rivers that were high in organics were high in phenolics
TOC (July, 1975)	-average = 8 mg/l
Humic and Fulvic Acids (July, 1975)	-average = 4 mg/l (90% of which was fulvic acid)
Tannins and Lignins (July, 1975)	-average = 0.4 mg/l

population requires more oxygen than the stream can supply, primarily due to the fact that ice cover precludes normal exchange of oxygen from the atmosphere.

Suggested Solutions to the DO Problem

Despite the fact that the DO problem of the Red Deer River has been well documented, the major cause has not yet been defined. The solutions to the low dissolved oxygen problem appear to be limited. The lowering of municipal and industrial waste loadings has been proposed however, there is a practical and technological limit to which this can be achieved. Considering, also, that this is not a major causative factor, increasing treatment efficiency may not be the answer.

Other suggested solutions include flow regulation to increase flows during the critical period and stream reaeration schemes.

Objectives

The objectives of this investigation were to assess and compare the role of biochemical oxygen demand in the dissolved oxygen budget of the Red Deer River system within regions not subjected to man-made organic loading, during periods of open water and ice cover. In order to carry out this investigation, two major factors have to be considered.

First, earlier studies (Baker *et al.*, 1975, 1977) could not explain the observed oxygen demand in terms of the

potential oxidation requirements of known biodegradable organic compounds found in the natural organic runoff. This left the generally more resistant humic and lignin-like substances as the most plausible causative factor. It was deemed necessary to design an experimental procedure to determine if significant biochemical oxygen demand could be attributed to this compound class because at normal river water concentrations the BOD can only be measured at a limited sensitivity using the standard BOD test (APHA, 1975).

Second, the standard BOD test which is carried out over 5 days at 20°C (APHA, 1975) does not confirm the possibility of significant oxygen demand at water temperatures approaching freezing. Therefore, it was also necessary to design the experiment to determine if the natural organic matter in the water does exert a measurable biochemical oxygen demand at a temperature approaching the winter, under ice, condition.

This experimental program was a laboratory investigation utilizing river water samples with only limited field investigations. The reproduction of a river environment within the confines of a laboratory may be an impossible task. However, in the true river situation the process(es) of interest may not be readily observed, due to masking by several other processes. This may not be the case in a controlled system. The information obtained from the laboratory investigation could then be used in future

studies to assess the true river situation through careful design and organization of an extensive field investigation.

II. LITERATURE REVIEW

Self-purification

The natural ability of a river to recover from a natural or man-made pollution load in the course of time, is termed self-purification (Klein, 1957). Self-purification is dependent to a large extent on biological reactions brought about by the activities (oxidation of organic compounds) of microorganisms (especially bacteria) under aerobic conditions. If conditions became anaerobic, the oxidation of organic compounds would result in obnoxious end products and many forms of aquatic life would be eliminated.

The rate of self-purification depends heavily on the amount of organic matter to be stabilized and its characteristics. McKinney (1956) pointed out that organic compounds containing a high proportion of oxygen, such as carbohydrates, are more easily stablized than those compounds with a lower proportion, such as fats and oils.

Self-purification is a complicated process and each river has its own capacity for purification which can only be assessed after an extensive chemical, physical, hydrological and biological survey.

Dissolved Oxygen

The dissolved oxygen budget is of primary importance to the health of a river. The substrate consumption rate and metabolic efficiency of the complete river biocenosis depends most heavily on the oxygen concentration in the

water (Wuhrmann, 1972). We must, however, keep in mind that certain organic and inorganic toxicants can affect metabollic activity despite the presence of dissolved oxygen. A chain of ecological reactions can occur in the river by a variance in the dissolved oxygen concentration, the extent of which depends on the duration and magnitude of the changes (especially their minima).

A river's dissolved oxygen concentration is controlled by four major processes (Engineering Methodology for River and Stream Reaeration, 1971):

1. Oxygen demand due to respiration by planktonic and benthic organisms, and chemical oxidation.
2. Oxygen exchange as a result of atmospheric reaeration.
3. Photosynthetic production of oxygen.
4. Oxygen contribution from ground water, surface runoff and storage.

In the first case, oxygen consumption by respiration (generally attributed mostly to microorganisms) is expressed as biochemical oxygen demand (BOD). Respiration can be represented in the following way:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{ENERGY} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

the rate of which is temperature dependent.

The uptake of oxygen through chemical oxidation has generally been assessed to be insignificant in river systems.

The second process is the primary natural oxygenating mechanism. The rate of oxygenation is highly dependent on temperature and stream turbulence. Under supersaturated conditions the oxygen transfer may be to the atmosphere. Table II-1 shows the effect of temperature on oxygen solubility.

The photosynthetic production of oxygen is the process by which carbohydrates are synthesized from carbon dioxide and water with the subsequent release of oxygen. This can be represented as:

$$\text{LIGHT ENERGY} + 6H_2O + 6CO_2 \xrightarrow{\text{chlorophyll}} C_6H_{12}O_6 + 6O_2 \quad \text{.....(2)}$$

The rate of oxygen production depends on the depth and duration of light penetration and stream temperature.

The fourth process, the oxygen contribution due to ground water, surface runoff and storage, is a site dependent contribution which can increase or decrease stream dissolved oxygen.

The general form for the oxygen balance equation in a natural stream has been developed from the classical works of Streeter and Phelps (1925). The Streeter-Phelps equations are based on only two major processes; (1) BOD and oxygen removal through oxidation of organic matter and, (2) oxygen replacement through reaeration at the surface. Taking the other possible processes mentioned above and expanding upon them, the dissolved oxygen profile along a stream can be

TABLE II-1. Dissolved oxygen saturation values in fresh water exposed to dry air containing 20.90% oxygen under a total pressure of 760 mm of mercury (adapted from Metcalf and Eddy, Inc., 1979).

Temperature, °C	Dissolved Oxygen, mg/l
0	14.62
1	14.23
2	13.84
3	13.48
4	13.13
5	12.80
6	12.48
7	12.17
8	11.87
9	11.59
10	11.33
11	11.08
12	10.83
13	10.60
14	10.37
15	10.15
16	9.95
17	9.74
18	9.54
19	9.35
20	9.17
21	8.99
22	8.83
23	8.68
24	8.53
25	8.38
26	8.22
27	8.07
28	7.92
29	7.77
30	7.63

represented by equation (3) (Engineering Methodology for River and Stream Reaeration, 1971):

$$\frac{\partial C}{\partial t} = D_L \frac{\partial^2 C}{\partial x^2} - U \frac{\partial C}{\partial x} + K_a(C_s - C) + P - K_c L - K_n N + A - S \quad \text{.....(3)}$$

where

A = rate of accrual of O_2 from drainage, ground water, etc. (ppm/day)

C = DO concentration (ppm)

C_s = saturation value of DC (ppm)

D_L = turbulent diffusion (dispersion) coefficient (ft^2/day)

K_a = aeration constant (l/day)

K_c = instream carbonaceous oxidation constant (l/day)

K_n = instream nitrogenous oxidation constant (l/day)

P = photosynthetic production rate (ppm/day)

S = benthal demand rate (ppm/day)

U = mean stream velocity (ft/day)

t = time (day)

x = distance along the stream (ft)

L = carbonaceous BOD (ppm)

L_a =uniform rate of addition of carbonaceous BOD (ppm/day)

N = nitrogenous BOD (ppm)

N_a = uniform rate of addition of nitrogenous BOD (ppm/day)

L and N are given by the solutions to

$$\frac{\partial L}{\partial t} = D_L \frac{\partial^2 L}{\partial x^2} - U \frac{\partial L}{\partial x} - K_c L + L_a \quad \text{.....(4)}$$

and

$$\frac{\partial N}{\partial t} = D_L \frac{\partial^2 N}{\partial x^2} - U \frac{\partial N}{\partial x} - K_n N + N_a \quad \ldots\ldots\ldots\

Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand is commonly defined as the amount of oxygen used by microorganisms while metabolizing or stablizing organic matter under aerobic conditions (Sawyer and McCarty, 1978). Wuhrmann (1972) stated that the biochemical removal of a compound from the environment involves its entrance into the food chain. The fate of these compounds may be either a permanent loss from the system due to oxidation in the energy metabolism or a change in distribution and chemical composition through the production of biomass. This process of organic matter removal is primarily attributed to bacteria.

A number of factors can readily affect the BOD profile in a stream . McKinney (1962) stated that changes within an aquatic environment may change the metabolic rates of microorganisms and therefore cause a wide variation in results. Some of the more important factors are; temperature, pH, the type and concentration of compounds in the stream, type of biomass, biomass distribution, biomass-water contact time, oxygen tension, and flow rate (Wuhrmann, 1972).

The biochemical oxygen demand exerted can be divided into two separate processes, the carbonaceous and nitrogenous oxygen demand. These processes generally occur at different rates and may or may not occur simultaneously.

First order kinetics has been most widely used to describe the relationship for a BOD reaction. However, the

BOD reaction rate is extremely complex, since it is dependent upon several parameters, which may vary from system to system. Therefore, it is difficult to assign a particular kinetic expression which best describes the course of the reaction. First order kinetics appear to fit, reasonably well, the course of a "simple" BOD reaction, which is attributed only to the carbonaceous demand (1st stage of a BOD reaction). The occurrence of nitrification can make the task of determining reaction rates virtually impossible (Young, 1973).

The oxidation of carbonaceous matter (organic carbon matter) is carried out by heterotrophic bacteria. These organisms require organic compounds as a source of carbon as well as a source of energy. They utilize such substances as carbohydrates and amino acids in addition to inorganic salts to build up their protoplasm (Klein, 1957).

Nitrification which brings about a nitrogenous oxygen demand, is a two stage oxidation process: (1) the oxidation of ammonia to nitrite; and (2) the oxidation of nitrite to nitrate. This process is carried out by a special group of autotrophic bacteria. Autotrophs obtain their carbon source from simple compounds such as CO_2, HCO_3^- and $CO_3^=$, and derive energy by the oxidation of such compounds as ammonia, sulfur compounds, ferrous compounds and hydrogen (Klein, 1957).

The carbonaceous and nitrogenous oxygen demand profiles in a stream can be determined by the use of equations (4) and (5). In general, most studies have indicated that the

carbonaceous component plays a more significant role in oxygen depletion of a stream. Wezernak and Gannon (1968) and Courchaine (1968) clearly demonstrated what many have suspected--that nitrification can be a very important mechanism in the removal of dissolved oxygen from streams. Actually, a generalization should not be made, since the relationship between, and the importance of, the carbonaceous and nitrogenous components in a stream are entirely dependent upon the stream environment. Nitrification is discussed separately in the next section due to its potential importance in river systems.

Drachev (1962) noted that oxidation of organic matter took place quite intensively in a U.S.S.R. river during ice cover and that organisms capable of growth at low temperatures increased progressively toward the lower reaches of the river. Similar observations were noted by Gordon (1970) in an Alaskan river during winter ice cover. Gordon mentioned that problems arise when the biochemical oxygen demand of municipal and industrial wastes are added to the natural requirement for dissolved oxygen. He examined the effect of added organic and inorganic nutrients and temperature and found little metabolic activity in a closed stationary river water system (BOD bottle), but rapid and extensive activity when certain organic nutrients were added.

Studies conducted by Ingraham and Stokes (1959) on psychrophilic bacteria (which are considered to be

ubiquitous in nature) indicated that at low temperatures, these organisms were capable of most biochemical activities observed at higher temperatures, but at a considerably reduced rate. Similarily, Prasad and Jones (1974) noted that degradation of various organic compounds by psychrophilic bacteria at 20°C and 2°C indicated that these organisms play an important role in the stablization of organic wastes in biological treatment systems at both temperatures. However, their activity was greatly reduced at 2°C. A good working definition of psychrophiles was given by Ingraham and Stokes (1959). They stated that, psychrophiles are bacteria that "grow appreciably and abundantly at 0°C within 2 weeks". Gordon (1970), based on earlier studies, points out that psychrophiles are present in river systems in sufficient quantities to play an important role in organic matter cycling.

In attempting to assess the degree of BOD in a river one should be aware of the dynamic nature of a river system. The method used to determine the BOD should be carefully examined in order to assess the reliability of the results obtained. The most widely used method is the standard bottle $BOD_{5,20}$ test (APHA, 1975). This closed, stationary method of assessing the BOD in a stream has been seriously questioned for several years. This test along with others are discussed in the final section of this literature review.

Nitrification

Nitrification is of great potential significance to the dissolved oxygen balance of streams. Many stream oxygen balance studies have been highly questionable because nitrification has been ignored as a deoxygenating factor in a stream and/or as a deoxygenating and rate constant distorting factor in BOD determinations (Blain, 1969).

The process of nitrification (biological oxidation of inorganic nitrogen) is carried out in an aquatic environment by autotrophic bacteria belonging to the family of Nitrobacteraceae. The principal genera in this family are Nitrosomonas and Nitrobacter which are widely distributed in nature and carry out the following oxidation reactions:

$$NH_4^+ + 1.5\ O_2 \xrightarrow{\text{Nitrosomonas}} 2H^+ + NO_2^- + H_2O \quad \text{.....(6)}$$

$$NO_2^- + .5\ O_2 \xrightarrow{\text{Nitrobacter}} NO_3^- \quad \text{.....(7)}$$

There are intermediates in the oxidation of ammonia to nitrite however, they are converted rapidly and there is very little accumulation.

These oxidation reactions derive energy which is used by the bacteria for carbon dioxide fixation for growth and cell synthesis. Through direct measurement experiments the following nitrogenous oxygen demand (NOD) was observed (Wezernak and Gannon, 1967):

$$NCD = 3.22(NH_3\text{-}N \rightarrow NO_2^-\text{-}N) + 1.11(NO_2^-\text{-}N \rightarrow NO_3^-\text{-}N) \quad \ldots\ldots\ldots (8)$$

Wezernak and Gannon (1967) suggested that the following reaction was occurring:

$$5CO_2 + NH_3 + 2H_2O \longrightarrow [C_5H_7NO_2] + 5O_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (9)$$

They indicated that the oxygen generated in this process must be subtracted from the theoretical amount required (equations (6) and (7)).

The oxygen requirement of over 4 mg of oxygen per milligram of ammonia nitrogen oxidized to nitrate nitrogen (equation (8)) indicates the importance of including nitrification in the dissolved oxygen balance, even when small quantities of ammonia nitrogen are present.

Nitrification has presented major problems in the measurement and interpretation of biochemical oxygen demand data. In the past it was assumed to occur after the carbonaceous oxygen demand. However, several investigators found that nitrification often occurs simultaneously with carbonaceous oxidation. It was then realized that many errors could be caused if nitrification was not controlled. A number of articles on methods of nitrification control have been published. For example, an article by Young (1973) describes chemical methods for nitrification control which are very simple and effective. Young and Baumann (1976) stated that nitrification control should be used as a

standard practice in BOD measurements conducted by any method and/or that supplemental nitrogen data should be determined to provide a measure of the impact of nitrogenous oxygen demand.

Several models have been developed to predict nitrification in streams, however, most investigators (for example: Stratton and McCarty, 1967; Wezernak and Gannon, 1968; and Courchaine, 1968) developed their models assuming that the nitrifying bacteria were suspended in the water column. It was pointed out by Stratton and McCarty (1967) and later emphasized by Finstein et al. (1978) that nitrification due to non-planktonic (stationary relative to streamflow, attached to streambed or sediments) organisms may be very important. That is to say, nitrification may be quite a significant factor in the benthic oxygen demand. Therefore, the location of the nitrifying bacteria, planktonic (in suspension) or non-planktonic, determines the type of stream model that would be appropriate. Studies conducted by Matulewich and Finstein (1978) indicated that in the Passaic River, nitrifying bacteria were several fold more abundant in both a mineral stream bed and in an organic ooze stream bed than in the water column.

The nitrogen balance in streams is very complex. It is affected not only by nitrification but also denitrification, N_2-fixation, ammonification and immobilization. Figure II-1 is a schematic diagram indicating the major transformations of nitrogen in a water-sediment system (Van Kessel, 1977).

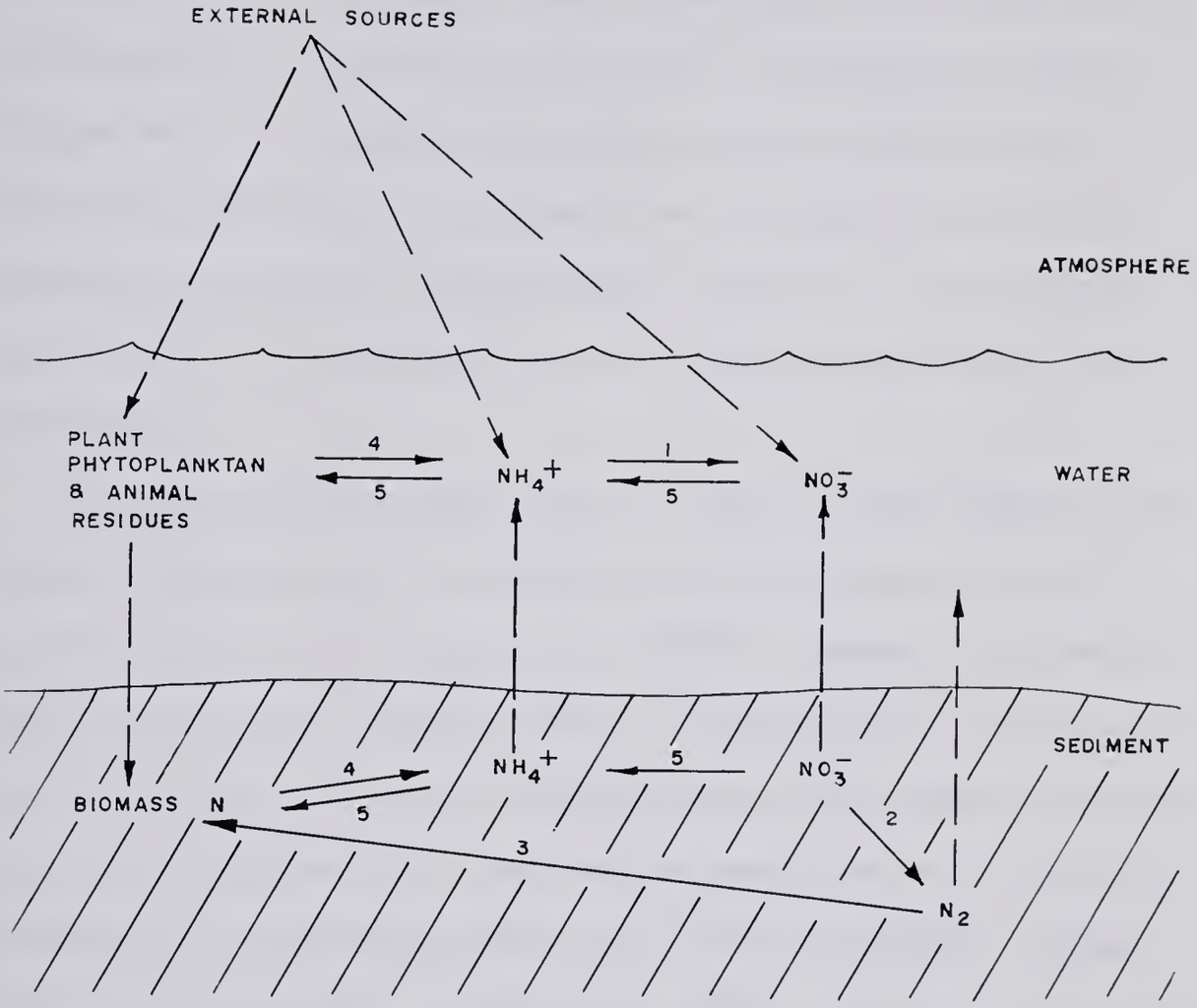


FIGURE II-1. Major transformations of nitrogen in a water-sediment system. (1) Nitrification; (2) denitrification; (3) N_2-fixation; (4) ammonification; (5) immobilization (adapted from Van Kessel, 1977).

These processes, in turn, are dependent on other variables such as temperature, DO and pH (Sharma and Ahlert, 1976). Matulewich and Finstein (1978) indicated that the absence, in field studies, of the NH_4^+ to NO_2^- sequence does not prove the absence of nitrification, since this could be masked by other nitrogen transformations, particularly ammonification and denitrification. Van Kessel (1977) pointed out that denitrification, which occurs under anaerobic conditons (as opposed to nitrification which requires an aerobic environment) occurs in the sediment and can occur in the anaerobic microzones of particles in suspension.

Nitrification occurs best at high temperatures. The optimum temperature for growth of nitrifying bacteria appears to be in the range of 28-36°C (Sharma and Ahlert, 1976). It has been found that as temperatures decrease the metabolic rates of the nitrifying bacteria decrease rapidly and it is expected that at temperatures below 4°C little or no growth of nitrifying bacteria occurs (Buswell *et al.*, 1954; Painter, 1970; Van Kessel, 1977). Studies conducted by Van Kessel (1977) indicated that at 4°C, denitrification occurred after a prolonged lag period whereas nitrification did not occur significantly in the sediment of a water-sediment system. However, there has been some evidence, from studies conducted on the North Saskatchewan River and in this investigation, to suggest that a significant amount of nitrification can occur in streams

below 4°C.

Humic Substances, Tannins, Lignins

Humic substances, tannins and lignins are complex natural organic compounds to which the yellowish-brown colour found in many natural waters is attributed. Humic substances are synthesized from plant residues whereas tannins and lignins are synthesized by plants and are major constituents of most plants. All of these compounds are ubiqiutous soil constituents and can enter the natural aquatic environment from the breakdown of aquatic plants. They have been studied mostly with respect to soils. However, it seems to be generally agreed that their physical and chemical properties are not significantly changed in the aquatic system. The exact chemical structures of these compounds have not yet been deciphered, in spite of interrationcal study for more than 100 years. Through comparisons of the biodegradability of these compounds with other organic compounds, they are considered to be relatively resistant to biological decay.

Tannins are considered to be polymers of gallic acid linked to glucose residues or polymers containing, at least in part, flavonoid nuclei (Baker *et al.*, 1975). Lignin is essentially a phenylpropane polymer with the definite presence of aliphatic hydroxyl and carbonyl groups (Hurst and Berges, 1967). The actual chemical structure of these compounds are unknown, they are generally described in terms

of extraction procedures. These compounds are considered highly resistant to biological degradation compared to most biologically synthesized compounds. Studies conducted by Sorensen (1962) indicate that several bacteria are capable of decomposing lignin however, this occurs very slowly when compared with most polysaccharides. Several investigators have suggested that the carboxyl derivatives obtained during lignin decomposition may play an important role in humus (humic substances) formation (Sorensen, 1962; Hurst and Burges, 1967; and Gjessing, 1976).

The distribution of tannins and lignins in the aquatic environment has not been well documented. Studies conducted by Telang et al. (1975) and Baker et al. (1975) indicated tannin and lignin concentrations of 10-735 µg/l in Marmot Creek and 100-1700 µg/l in the Red Deer River drainage basin, respectively.

Humic substances (also called humus) are categorized into three groups of compounds based on extraction procedures: (1) humic acid, the fraction soluble in an alkaline solution but insoluble in acidic solutions; (2) fulvic acid, soluble in acidic and alkaline solution; and (3) humin, insoluble in acidic and alkaline solutions. Most of the literature concentrates on the humic and fulvic acid fractions, since they appear to be most abundant.

Humus is synthesized through a process called humification, which is not entirely understood. Carbohydrates, proteins, tannins and lignins are considered

to be the most important starting materials in this process (Gjessing, 1976). Humus is not a definable organic compound. However, it has been described as being made up of highly complex phenolic polymers and is of high molecular weight (anywhere from a few hundred to several thousand) (Hurst and Burges, 1976). Flaig (1975) describes humus as a dynamic system which continually changes as its constituents decompose and are formed anew. Several factors, such as climate and soil conditions affect this diverse process. As in the case of tannins and lignins, humus is considered to be highly resistant to bio-degradation. Gjessing (1976) indicated that the standard $BOD_{5,20}$ test is not a useful method of characterizing aquatic humus since this method gives low and variable results (ie: Humus coloured water containing 6.3 mg/l of organic carbon has a $BOD_{5,20}$ in the range of 1 mg-O_2/l).

Several methods have been investigated for the characterization of humic substances. These include elementary and functional group analysis, UV, visible and IR spectroscopy, thermal analysis, gas chromatography-mass spectrophotometry (GC-MS), gel chromatography and a relatively new technique using high pressure liquid chromatography (HPLC).

Despite the geographic location in which humus is formed, there appears to be similarities in each fraction with respect to their chemical and physical properties. Permanganate oxidation products of humus from various soil

samples were examined by Khan and Schnitzer (1972) and they concluded that the skeletal structure of each fraction was essentially similar and that the chemical structures of humic and fulvic acids were more closely related to one-another than to humin.

The type of compounds determined from examination of each fraction of humus is dependednt upon the method of purification. It appears that the compounds identified in aquatic humus are the same as soil humus, although the relative compositions may be different (Gjessing, 1976). The primary elements in humus are carbon, hydrogen and oxygen and the major functional groups are carboxyl, hydroxyl and carbonyl groups (Rashid and King, 1967). Schnitzer (1972) indicates that the carbon content of humic acids range from 50-60%, oxygen content from 30-35%, hydrogen and nitrogen from 4-6% and 2-6%, respectively. He also pointed out that fulvic acids have lower carbon content, 40-50%, and higher oxygen content, 44-50%, than humic acids. According to other published data, soil humus is composed of 45-63% carbon, 3-6% hydrogen, and 0.5-5% nitrogen (Gjessing, 1976). Gjessing (1976) states that aquatic humus generally has a lower carbon and nitrogen content but a higher content of hydrogen (on the average 43%, 1.1% and 5.5%, respectively).

It is generally accepted that humic substances are capable of scavenging several compounds and elements. For example, there have been indications that humic acids can irreversibly absorb ammonia, nitrite and amino acids under

oxidative conditions (Sorensen, 1962; Steelink, 1963; and Rashid and King, 1969). Also, iron is always found to be combined with aquatic humus. The way in which humus binds or combines with these elements and compounds has yet to be determined. Schnitzer (1972) points out that it has been postulated that fulvic acid, has at least in part, a polymeric, sponge-like structure of phenolic and carboxylic acids, and this structure can absorb inorganic and organic compounds of proper molecular dimensions on its outer surface and internal voids.

It has been shown that the colour of humus water can be correlated to other chemical parameters and elements. There is a close relationship between color and chemical oxygen demand (COD), between color and organic carbon, and between colour and iron (Gjessing, 1976). It is also interesting to point out that Gjessing (1976), based on his earlier investigations, observed that the higher the colour (or carbon content), the lower the organic nitrogen.

The concentration of humic substances in natural waters varies both geographically and seasonally. Prakash *et al.* (1975) indicated that the values normally range from less than 1 mg/l to 20 mg/l, but in some dystrophic bog lakes and ponds very high concentrations may be detected. Baker *et al.* (1975) reported values of 0.9 to 9.5 mg/l of humic and fulvic acids in the Red Deer River basin, 90% of which was in the form of fulvic acids.

The Measurement of Biochemical Oxygen Demand (BOD)

In order to reliably measure the biochemical oxygen demand in a water system (lake, stream or treatment process) it must be realized that the method employed must be capable of meesuring the cumulative effect of the following parameters present; the population and species of microcrganisms, the type of substrate, the concentrations of substrate and interfering substances and, the physical characteristics of the water system examined. It must be stressed that a BOD test is only an indicator of the extent and rate of bio-availability of organic material present in the water and not a method of quantifying organic concentrations (Clark, 1974).

The most widely used method of measuring the BOD is the standard bottle, $BOD_{5,20}$ test (APHA, 1975; and Sawyer and McCarty, 1978). This method generally involves incubating a sample with a predetermined dissolved oxygen (DO) level for 5 days at 20°C in the dark and then measuring the remaining DO. The sample is either diluted or undiluted (depending on the amount of oxygen that is expected to be utilized), contains a microbial seed (if the sample does not contain suitable microbial population), and contains essential nutrients for the microorganisms. Corrections for dilution and microbial seed are taken into account in the amount of oxygen utilized. The principle of this BOD test is sound. However, there are several limitations associated with this technique, namely the pretreatment of the sample, the static

nature of the test, and the arbitrarily chosen 5 day duration of the test. Gaudy (1972) discusses, at length, the limitations of this test method and the incorrect assumptions which have been made in the interpretation on the data. The precision of this technique is considered to be about ±20 percent (Clark, 1974).

There are several simple refinements that can be made to the standard BOD test. It is not necessary to measure BOD only in terms of 5 days or to obtain just one value on the BOD curve. Clark (1974) points out that by setting up several simultaneous standard BOD tests and analyzing them at particular intervals over a chosen number of days one can obtain a clearer picture of the rate and extent of biochemical oxygen demand. Further refinements are offered by continuous or semi-continuous monitoring systems. These can range from inserting a self-stirring DO probe in a standard BOD bottle to the various types of respirometers.

Respirometric techniques have been gaining in popularity over the years and it has often been suggested that these methods might be used in the place of the standard bottle test. Respirometers are devices which monitor oxygen uptake within a system, containing an excess supply of oxygen, by the application of manometric techniques. One of the critical requirements for a respirometer is the ability to absorb the carbon dioxide gas generated through biological respiration. There are several designs of respirometers most of which are based on the

"Warburg" design. However, electrolytic respirometers have been gaining in popularity over the past 19 years. Montgomery (1967) discusses many of these methods in his review paper on respirometric techniques. Tuffey (1974) indicated that "Warburg" respirometry was poor for application in low level BOD studies and inappropriate for nitrification studies on stream waters (due to alkali inhibition which is certainly related to sample size) however, quite appropriate for high level carbonaceous BOD studies.

The electrolytic respirometer was developed by Clark (1960) and the final form of the apparatus was described by Young et al (1965). A slightly modified version of this system is represented in Figure III-4. The electrolytic respirometer continuously and automatically maintains the oxygen pressure within the 1 litre reaction vessel by supplying the sample with oxygen produced from the electrolysis of water in a weak electrolyte. The current passing through the electrolysis cell is proportional to the oxygen consumed by the sample and may be continuously monitored. Carbon dioxide is absorbed by a KOH reservoir fitted to the neck of the reaction vessel. When the pressure in the apparatus falls, owing to oxygen uptake, the level of the electrolyte changes and an a.c. circuit is broken. This causes current to pass through the platinum electrodes resulting in the generation of oxygen. Oxygen generation ceases when the oxygen pressure is restored to its original

value.

Intensive studies have been done by Young and Baumann (1972) on the implementation of the electrolytic respirometer. They stated that an electrolytic respirometer provides a more direct and continuous measure of BOD than the standard bottle test or other respirometer methods. This does not imply that it is more accurate, since there are no standards to compare with. The greatest advantages of such a system are that larger and more representative samples can be analyzed (and therefore a large amount of sample is available for further analysis at the end of a run) and that the BOD precision appears to be greater than other methods. One of the major sources of error in the electrotytic respirometer method, is attributed to barometric pressure changes. A barometric pressure increase results in a positive error, however, this error can be corrected when the results from a blank are taken into account. A decrease in barometric pressure retards the cell operation, which results in a negative error. Corrections for a pressure decrease can be "estimated" through calculations. Another source of error may be due to possible effects of the high dissolved oxygen concentrations in the sample (since the vessel is purged with relatively pure oxygen). High dissolved oxygen levels may have an effect on the microorganisms and there may be the possiblility of dissolved oxygen reacting with other compounds in solution.

In general, the advantages of respirometric methods

over the standard bottle test are; that the conditions of nature may be simulated more closely in a respirometer than in a BOD bottle, that a more or less continuous measurement of oxygen uptake may be obtained, that the effect of various factors on the oxygen uptake may be conveniently studied and that often useful information can be obtained in less than five days (Montgomery, 1967).

BOD results used in conjunction with total organic carbon (TOC) results can give a descriptive profile of a stream or any other water system in terms of specific concentrations of organics, the rate at which these organics decompose and the oxygen required for stablization and the portion of organic material which will remain inert. It is evident, that with the development of more precise methods of measuring BOD and a greater understanding of the process will lead to better utilization of BOD information in water quality studies.

III. EXPERIMENTAL DESIGN

Sampling

Four potential sample sites in the Red Deer River basin were chosen based on earlier investigations (Baker *et al*, 1975; and Red Deer River Flow Regulation Planning Studies, 1975). These sites were examined in the spring of 1978 to determine their TOC concentrations (TABLE III-1). A sampling site on the Blindman River near Blackfalds at highway 2A (Figures I-1 and III-1) was chosen for this investigation based on the relatively high TOC level and accessibility.

The Blindman River, although it is confluent to the Red Deer River downstream of the city of Red Deer, is representative of the major tributaries upstream of Red Deer. It is 130 km long and drains an area of 1787 km^2 (Kellerhals *et al*, 1972). The major vegetative communities of this drainage area consists of grassland with aspen groves, aspen parkland and boreal-cordilleran spruce forest transition (Baker *et al*, 1977). A considerable portion of the soil in this area is muskeg and much of the land is cultivated.

The Blindman River has a long term mean annual discharge of 3.3 m^3/s. The channel bed is shallow gravel over moderatelly erodible shale. The water has a characteristic yellowish-brown colour due to muskeg leaching.

Grab samples (50 litres) were collected approximately twice a month from July, 1978 to January, 1979. The

TABLE III-1. Total organic carbon (TOC) measurements at four potential sampling sites: May 31, 1978.

Site	TOC, mg/l
Blindman River near Bentley at highway #51	16.8
Blindman River near Blackfalds at highway #2A	19.2
Red Deer River west of Innisfail at highway #54	7.1
Medicine River at highway #54	15.6

FIGURE III-1. Sampling site on the Blindman River near Blackfalds, December, 1978.

temperature and degree of ice cover were noted on each visit. Flow rates were obtained from Water Survey of Canada based on a flow monitoring station just downstream from the site (Figure III-2). The samples were held at 4°C prior to treatment and/or analysis.

Oxygen Demand Experiments

In order to assess the oxygen demand associated with natural organic constituents in the Blindman River it is necessary to perform a mass balance on the major constituents of the biochemical oxygen demand "reaction". However, the $BOD_{5,20}$ normally occurring within these streams are of the order of 1 mg/l, which suggests that the organic carbon utilized is very low (1-2 mg/l was anticipated). These two factors play an important role in the experimental design because: (1) the reliability of the results obtained using the Standard BOD test or respirometric methods could be seriously questioned when working at such low levels; and (2) the objective of performing a mass balance to confirm the nature of the oxygen demand is limited to the sensitivity and reproducibility of the TOC procedure (1-2 mg/l). Sample concentration, to increase the organic matter concentration, was considered to be a viable solution to overcome the limitations. Also, in order to assess the kinetics of the biochemical oxygen demand a continuously monitored electrolytic respirometer was employed for the BOD studies.

FIGURE III-2. Flow monitoring station downstream from the sampling site.

The major parameters measured in this investigation were pH, total alkalinity, total organic carbon (TOC), inorganic carbon (IC), nitrite nitrogen, nitrate nitrogen, ammonia nitrogen and total kjeldahl nitrogen (TKN). These parameters were measured within 24 hours of sample collection, at the end of the concentration procedure, at the end of an electrolytic respirometer run and, occasionally, some of the parameters were measured during a run. Samples were also analyzed by high pressure liquid chromatography (HPLC) in order to assess its potential as an investigative tool in aquatic humus studies.

Sample concentration was accomplished using a rotary evaporator (Buchi Rotovapor-R) at a temperature not exceeding 50°C. It took approximately 6 days to concentrate a sample 7-8 times (by volume) to obtain approximately the 4 litres used in each run. The concentrate was filtered through acid washed, 0.45 μ Millipore membrane filters and then stored at 4°C. When enough concentrated sample was obtained for one run, the chemical parameters were measured. The concentrations of the major dissolved constituents before and after concentration were then examined to evaluate the overall sample change through the concentration step. This also allowed the calculation of a concentration factor for each individual component of interest, in addition to the overall volume concentration factor.

Concentration by freeze drying was initially considered but was found to be impractical for the quantities of sample

required. The obvious major limitation to the concentration method used would be the loss of volatile organic materials and dissolved gases. Structural changes of the dissolved organic compounds (such as humic and lignin-like materials) were judged to be minimal at temperatures below 50°C.

An alteration of the relative concentrations of the components in solution and/or an increase or decrease of absolute parameter concentrations through the concentration procedure may have serious implications on BOD exertion. For example, the inorganic ion concentration would increase through the concentration procedure, potentially causing inhibition or stimulation of microorganisms. It was therefore necessary to examine the effect of concentration on BOD exertion. This was done by performing standard bottle $BOD_{5,20}$ tests (APHA, 1976) using a primary effluent seed on a sample concentrated by factors of 2.0, 5.0 and 6.3 to compare with an unconcentrated sample. Also, in order to investigate the effect of concentration on the rate of oxygen uptake, a semi-continuously monitored standard bottle BOD test was performed on a concentrated and unconcentrated sample containing a primary effluent seed. This was accomplished by inserting self-stirring DO probes (YSI model 5720) into the BOD bottles until all available DO was utilized.

An electrolytic respirometer was considered as the best method for this type of an investigation. An electrolytic respirometer can continously monitor the oxygen demand, it

can be readily modified for sample analysis during an experiment and it can use more representative sample sizes. The electrolytic respirometer used was the A.R.F. Products Inc., Model ER 101 equipped with a modified reaction vessel (Figures III-3 and III-4). The reaction vessel was redesigned to allow small aliquots to be removed for analysis during sample runs, as well as to allow the insertion of standardized mineral cores coated with microbial slime, cultivated in the Blindman River.

The electrolytic respirometer oxygen demand experiments were set up using four 1.3 litre reactors, three sample reactors and one control reactor containing distilled water plus seed. It was necessary to provide a seed in these experiments since there are very few organisms in suspension in the river water column, most are sessile (Baker et al, 1977). The seed was provided by two methods. In the first instance, in an attempt to roughly reproduce the river situation, a microbial slime seed was obtained by placing igneous rock drill cores (3.2 cm diameter x 2 cm thick) in a rack on the bed of the Blindman River for 1 to 2 weeks. Two of these cores were then placed in each reactor by means of a central ground glass joint. Inside the reactor, the cores were supported on a glass rack over top of a magnetic stir bar (Figure III-5). Due to problems encountered with the use of the seed cores, an injection of settled primary effluent was used as another method of providing a microbial seed.

Each respirometer run was carried out in the dark at a

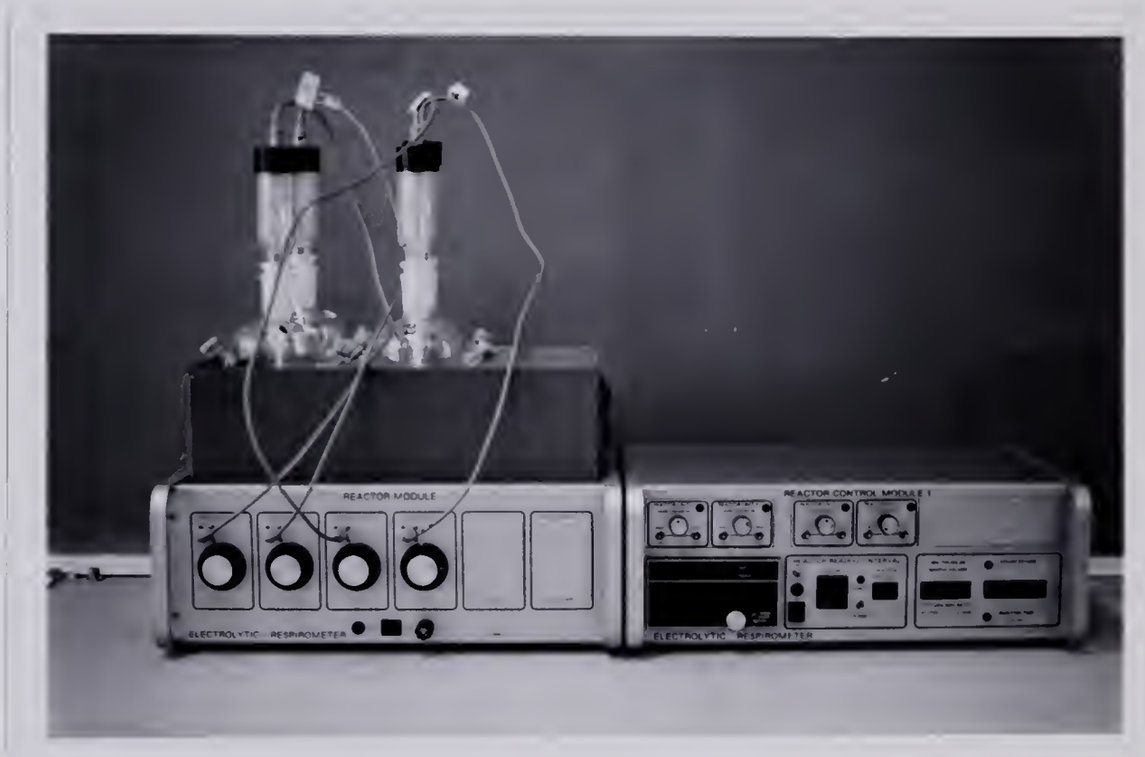


FIGURE III-3. Electrolytic Respirometer
(A.R.F. Products Inc., Model ER 101)

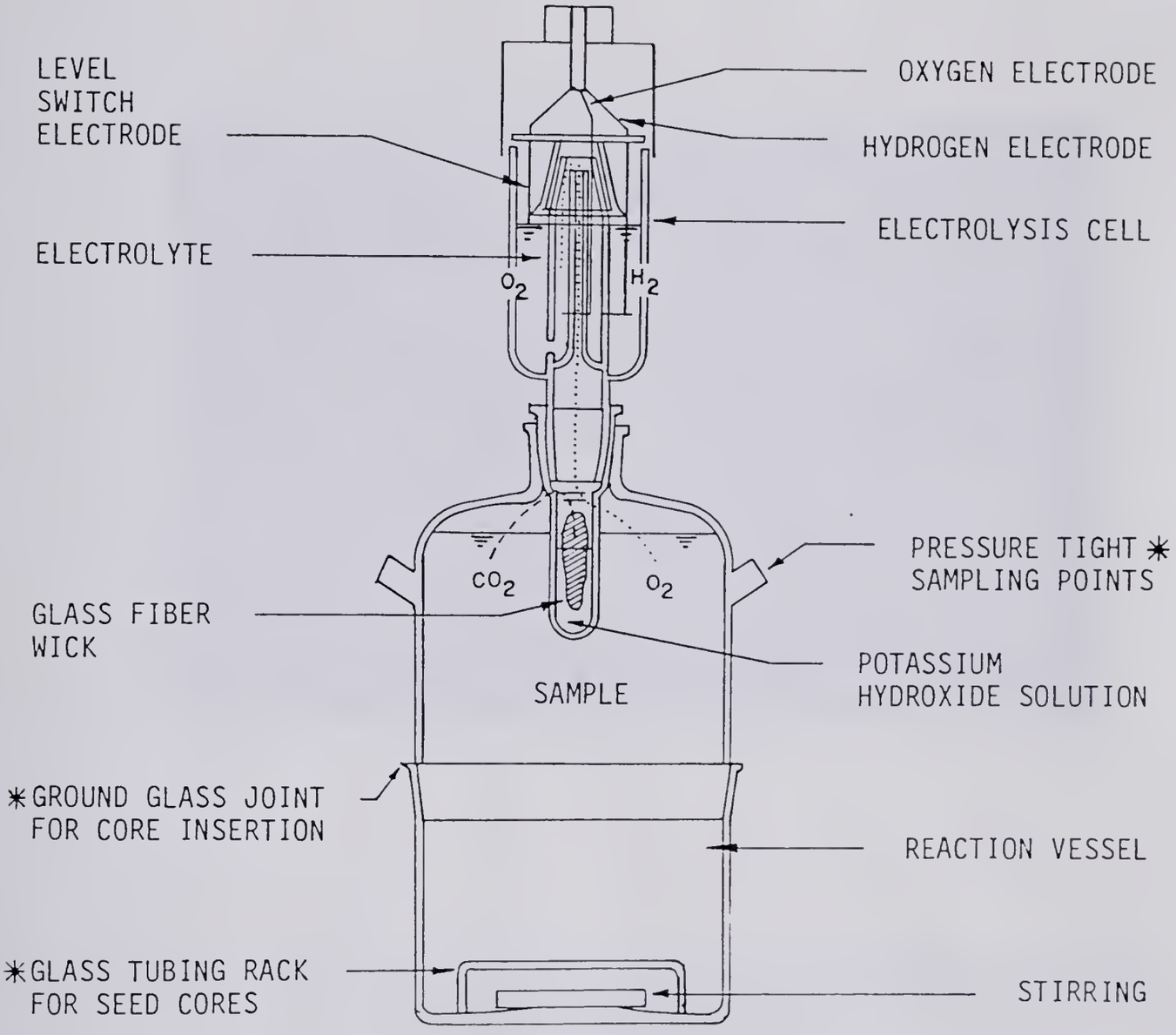


FIGURE III-4. Modified electrolytic respirometer reaction cell. (* modifications made to the original design by Young et al, 1965).

FIGURE III-5. The seed cores, glass rack and magnetic stir bar which were placed at the bottom of the electrolytic respirometer reaction vessel.

controlled temperature of either 20°C or 4°C for over 200 hours. During the course of a run, a 10 ml sample was extracted from one of the two septa mounted on the reactor while an equal volume of distilled water was injected in the second septum. The extracted sample was filtered through an acid washed, 0.45 μ Millipore filter and analysed for nitrite and nitrate. At the end of the run, the samples were filtered and all of the parameters were measured. For the runs utilizing seed cores the biomass was estimated by air-drying the cores, weighing, washing, air-drying and reweighing. This was done to provide a control seed correction in terms of BOD per mass of biomass since slime consistancy, between cores, could not be controlled.

The electrolytic respirometer runs were primarily performed on untreated concentrated samples, although one run was performed, at 20°C, on a concentrated sample containing a nitrifying inhibitor (5 mg/l of 1-Allyl-2-Thiourea, Eastman Kodak) in an attempt to measure only the carbonaceous oxygen demand. Also, a run was performed, at both 20°C and 4°C, on an unconcentrated sample in order to establish the need for sample concentration.

Field investigations were limited to comparing the parameters measured in fresh unconcentrated samples from each sampling trip. However, on one occassion the parameters measured from fresh unconcentrated samples obtained from the site were also compared with samples obtained (2 hr later) 6.4 km downstream, near the mouth of the river (Figure

III-6).

Analytical Methods

All concentrated samples were first filtered through acid washed, 0.45 μ Millipore membrane filters prior to any analysis. This was done to remove suspended inorganic precipitate and biomass so that only dissolved parameters would be measured. Also, all dilutions and solutions were prepared using purified water from a Milli-RO and Milli-Q purification system (Millipore Co.).

The TOC and IC were analyzed by the combustion infrared method 505 (APHA, 1975) using a Beckman model 915A Total Carbon Analyzer and a Beckman model 865 Infrared Analyzer. The maximum deflection of the chart recorder was set at 100 mg/l carbon for a 20 μl sample injection. All concentrated samples were appropriately diluted before analysis. TOC was measured by acidifying the sample to pH 2 with 50% HCl and purging with nitrogen gas just prior to injection. All samples were frozen if they were not analyzed the same day. Prior to analysis 20, 50, 80 and 100 mg/l carbon standards were run. The precision of this method approaches 1 to 2% or 1 to 2 mg/l carbon, which ever is greater.

The pH of each sample was measured with a glass combination electrode and a Hach portable pH meter which was standerdized with pH 4.0 and pH 9.0 buffers. Following each pH measurement, the total alkalinity was determined. This

FIGURE III-6. Mouth of the Blindman River, 6.4 km downstream from the sampling site.

was measured by titration of a 50 or 100 ml sample (depending on the suspected level of alkalinity) with 0.02N sulfuric acid to a pH 4.5 endpoint according to method 403 (APHA, 1975). The endpoint was detected using the glass electrode and pH meter.

Nitrite levels were determined by spectrophotometrically measuring the reddish purple azo dye produced by the reaction of nitrite with sulfanilamide (4-amino-amide-benzenesulfonic acid) in a highly acidic solution which is then coupled with N-1-(naphthyl)-ethylenediamine dihydrochloride (according to method 420 APHA, 1975). Nitrite was measured the same day the sample was obtained, using a 50 ml (filtered) sample. Each sample was reacted with 1 ml of sulfanilamide solution (2-8 min) followed by 1 ml of 1-N-(naphthyl)-ethylenediamine dihydrochloride solution (10 min - 2 hr). The absorbance was measured through a 1 cm path length at a wavelength of 543 nm, using a Spectronic 20. Nitrite nitrogen concentration was determined from a calibration curve (0-100 µg/l nitrite nitrogen) which was checked daily using fresh standard nitrite solutions.

Nitrate analysis was performed with a modified version of the cadmium-copper reduction method of Strickland and Parsons (1972). In this method nitrate is reduced quantitively to nitrite. The cadmium-copper column was prepared using a 50 ml buret one third filled with granular purified cadmium (Fisher Scientific Co.) pretreated with a

solution of copper sulfate. The column was then rinsed and stored under dilute ammonium chloride. 50 ml of sample (with 1.5 ml dilute ammonium chloride) was passed through the column twice at about 2 ml/min, with 10 ml being collected for nitrite analysis and measured as in the above method with appropriate reagent volume adjustments. Samples were filtered and analyzed the same day they were obtained. Column efficiency was checked after every few samples using standard nitrate solutions. The reduction efficiency was maintained at 80-100%. Nitrate nitrogen concentration was determined from the nitrite nitrogen calibration curve (0-100 µg/l nitrate nitrogen) which was checked daily and appropriate corrections were made for column efficiency, initial nitrite and dilution.

The ammonia nitrogen analysis was done acidimetrically in accordance with method 418A & B (APHA, 1975). Analysis was done on fresh 500 ml samples which were buffered at pH 9.5 with a borate buffer. A dechlorinating agent (sodium arsenite) was also added to the solution. The samples were then distilled into a boric acid indicating solution (purple) which turned green upon absorption of ammonia. The amount of ammonia absorbed was determined by a back titration with 0.02N sulfuric acid (to a purple endpoint) and application of the necessary blank correction.

Total Kjeldahl Nitrogen (organic nitrogen) was analyzed by the digestion, distillation procedure using titrimetric detection in accordance with method 421 (APHA, 1975).

Samples which could not be analyzed immediately were preserved with 0.8 ml/l of sulfuric acid. Since ammonia levels were very low, ammonia removal prior to analysis was deemed unnecessary. 50 ml of a sulfuric acid-potassium sulfate digestion reagent (containing a mercury catalyst) was added to 500 ml of filtered sample and then digested (about 4 hours). The digested sample was cooled, diluted with about 300 ml of ammonia free water and then the pH was adjusted to above 7 with 50 ml of sodium hydroxide-sodium thiosulfate reagent. This solution was then distilled into a boric acid indicating solution and back titrated, as in the ammonia analysis. A blank was carried through all of the steps daily and the necessary correction was applied to the results.

Dissolved oxygen was measured with a polarographic probe (YSI model 5720 or 5750) and a YSI model 54RC dissolved oxygen meter.

HPLC has only recently been studied as a potential technique for the investigation of humic substances. A standard method has not been devised using the HPLC, so this investigation was based on one set of crudely chosen chromatographic parameters determined to give a reproducible chromatogram with the river samples. The HPLC work done in this investigation involved chromatogram comparisons between concentrated and unconcentrated river samples before and after each run. A chromatogram of a fulvic acid standard was also compared with a concentrated river sample. The standard

was prepared by dissolving technical grade Humic acid (Aldrich Chemical Co. Inc.) in an alkaline solution, then acidifying to pH 2 and filtering (the final TOC was about 40 mg/l). In the preparation of the standard, the "humic acid" prepared by Aldrich Chemical Co. was actually assessed to be "humus" (a combination of humic acid, fulvic acid and humin). A large fraction of this material was insoluble in KOH and upon acid treatment, a small amount of precipitation occurred leaving behind a highly coloured solution, presumably fulvic acid.

The chromatograms were obtained using model 6000A pumps, a model 660 solvent programer and a μ Bondpak C_{18} column (3.9 mm dia. x 30 cm length), all produced by Waters Associates Inc., with a Vari-Chrom UV-VIS detector, produced by Varian. The pumps were run at a pressure of about 1000 psi using an acetonitrile/water solvent system. All samples were analysed at a wavelength of 254 nm.

IV. RESULTS

Physical and Chemical Properties of The Blindman River

The physical and chemical properties of the Blindman River over the duration of the sampling program are presented in Table IV-1.

The river flow rate rose from a low of less than 0.1 m^3/s in the summer to 1.2 m^3/s in October. The flow rate was seen to decrease as ice cover was established. The first complete ice cover occurred between November 10 and 23 and by January 4, 1979 the ice cover was approximately 60 cm thick.

The pH was relatively constant near 8.3 until it began dropping to less than 7.8 during cold water temperatures and during ice cover. Total alkalinity initially dropped from summer to early fall as flow increased but subsequently rose as ice cover became established. These changes are likely due to increased bicarbonate concentrations and are reasonably well reflected in the IC values.

Throughout the sampling period the total organic carbon concentration remained relatively constant (at 16 mg/l). Likewise, TKN and nitrite levels remained relatively constant at 893 and 2 µg/l-N respectively, although nitrogen analysis was done only in the later half of the sampling period. The TKN was found to range from 5.2 to 6.7% of the TOC with a mean value of 5.9%. Nitrate levels increased dramatically from 9 µg/l-N in October to 142 µg/l-N in January. Based on the method used for ammonia nitrogen

TABLE IV-1. Physical and chemical properties of the Blindman River near Blackfalds: July 1978 to January 1979.

Date	Water Temp. °C	Ice Cover cm.	Flow Rate m^3/s	pH	Total Alkalinity mg/l as $CaCO_3$	IC mg/l as C	TOC mg/l as C	TKN µg/l as N	NO_2^- µg/l as N	NO_3^- µg/l as N	DO mg/l	BOD 5,20 mg/l
July 13	17	nil	0.1	8.3	204	50	17	-	-	-	-	-
July 24	17	nil	0.1	8.4	202	44	17	-	-	-	-	-
Aug. 5	17	nil	0.1	8.3	205	42	16	-	-	-	-	-
Aug. 23	15	nil	0.1	8.2	180	46	14	-	-	-	-	-
Sept. 1	19	nil	0.2	8.3	182	42	15	-	-	-	-	-
Oct. 4	7	nil	1.2	7.7	179	37	18	-	-	-	-	-
Oct. 22	4	nil	1.0	7.4	223	48	17	850	-	-	-	-
Oct. 31	1	partial	1.0	7.8	228	54	14	920	0	9	-	-
Nov. 10	0.5	partial	0.9	7.8	225	39	13	660	2	2	-	-
Nov. 23	0	30	0.6	7.5	299	72	17	950	2	25	-	-
Dec. 14	0	30	0.5	7.7	318	79	15	1000	2	74	7.9	2.2
*Dec. 14	0	30	-	7.7	320	73	14	900	2	100	9.5	1.8
Jan. 4	0	60	0.5	7.3	389	90	15	980	2	142	4.1	-

*data obtained 6.4 km downstream from site

analysis, which has a sensitivity to 50 µg/l-N, no ammonia nitrogen was found in the samples subjected to nitrogen analysis.

Dissolved oxygen values measured on site in December and January were 7.9 and 4.1 mg/l respectively. These values are well below saturation at ambient water temperatures for this time of the year.

A comparison of the physical and chemical properties of the Blindman River at the chosen sampling site and 6.4 km downstream (near the mouth of the river), on December 14, are also shown in Table IV-1. The travel time for a slug of water to flow this 6.4 km stretch was estimated (assuming constant flow) at about 4 hours. However, it was observed that the flow at the downstream site was substantially greater than the upstream site. Along this stretch the most significant changes occurred in TKN, nitrite nitrogen and dissolved oxygen. TKN decreased by 100 µg/l-N and nitrate increased by 26 µg/l-N. The dissolved oxygen increased from 7.9 to 9.5 mg/l. There was also a slight change in the $BOD_{5,20}$ (using a primary effluent seed), which was seen to decrease by 0.4 mg/l (2.2 to 1.8 mg/l, average of three tests) and a corresponding decrease in TOC of 1 mg/l, however, considering the analytical methods used and the erratic nature of the standard BOD test at low concentrations, these decreases are not considered very significant. The IC was also seen to decrease however, this decrease may also be questionable considering the

insignificant change observed in total alkalinity.

Concentration Procedure

Figure IV-1 illustrates the relationship between samples of varying concentration (based on volume reduction) and their exerted BOD (standard BOD test over 4.5 days at 20° C using a primary effluent seed). The two curves represent the actual BOD measured and the BOD values normalized back to the original sample concentration by dividing through by the volume reduction factor (each data point represents the average of three tests). It is seen that a concentration factor of 2 causes the greatest effect on BOD exertion with higher concentration factors having a lesser effect. Corresponding to this is the observation that no precipitate occurred over the incubation period in the sample concentrated 2X, but an inorganic precipitate did occur at the higher concentration factors.

The effect of concentration (8.3X, based on liquid volume reduction) on the rate of DO uptake is illustrated in Figure IV-2. The lag times are not shown in this figure, however, it was noted that the concentrated sample had a shorter lag time than the unconcentrated sample (15 hours versus 37.5 hours). From Figure IV-2, it is seen that the oxygen uptake occurs in one continuous stage in the concentrated sample, whereas it occurs in two distinct stages (separated by about a 15 hour lag phase) in the unconcentrated sample.

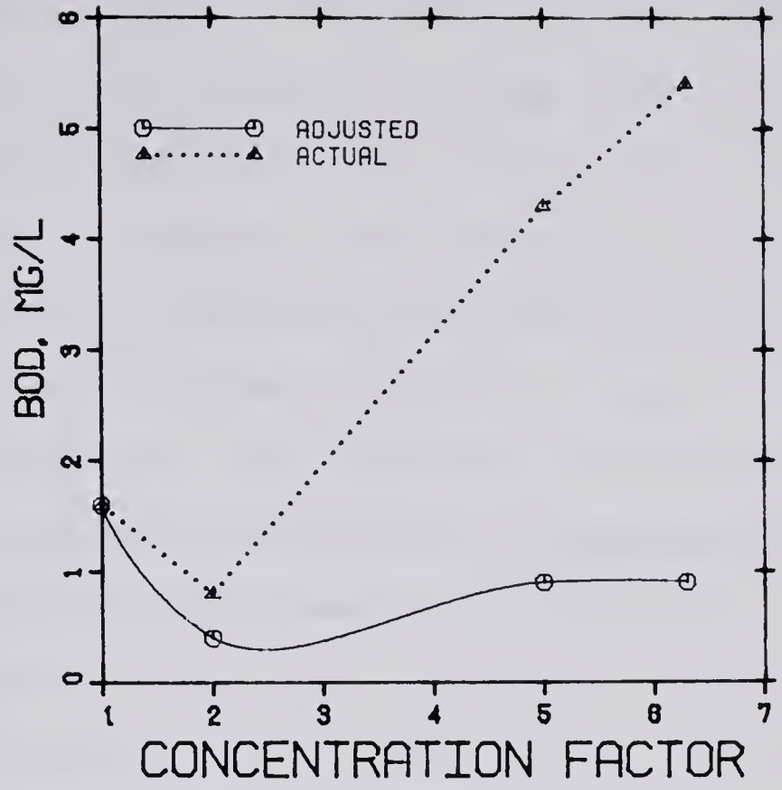


FIGURE IV-1. Effect of various sample concentrations on biochemical oxygen demand (measured at 4.5 days).

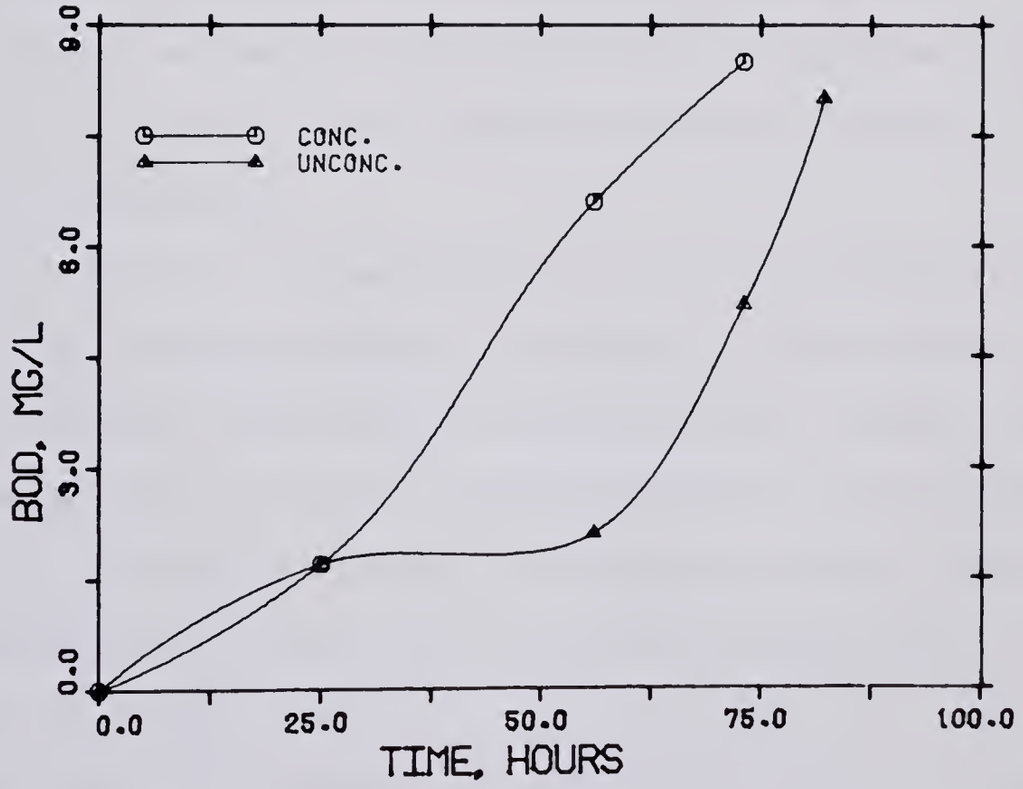


FIGURE IV-2. Oxygen demand curves of a concentrated (8.3X) and an unconcentrated river sample (obtained using the standard bottle BOD test, seeded and corrected for blank).

The overall sample change, with respect to the major dissolved constituents, through the concentration step are shown in Table IV-2. Included in this table is the mean value and the standard error of estimate of the mean value of each component loss and the sample concentration factor. Losses in IC, total alkalinity and TKN were quite large however, losses in TOC were minimal (average of 6.1%). In some cases there was a net increase in TOC however, these values were rejected on the basis of suspected contamination such as, contaminated glassware and adsorption of volatile organics from the laboratory environment prior to analysis. The lack of precision of the TOC test can also be questioned, however, it was considered that it would be more appropriate to report a more conservative loss by rejecting those values indicating a net increase. During the concentration procedure much inorganic precipitate occurred which may account for the roughly equivalent losses in IC and total alkalinity.

The effect of concentration on nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N) are shown in Table IV-3. Nitrite nitrogen was entirely removed in the concentration procedure however, nitrate nitrogen behaved erratically. Samples containing low levels of nitrate nitrogen increased upon concentration where as samples containing high nitrate nitrogen decreased.

The effect of concentration on pH is shown in Table IV-4. The pH of samples before and after concentration did

TABLE IV-2. Sample concentration factors and apparent losses for the major dissolved constituents during concentration.

Date	Concentration factor based on: liq. vol. removal	increase in TOC	increase in IC	increase in Tot. Alk.	increase in TKN	Percent loss with respect to liq. vol. concentration: TOC	IC	Tot.Alk.	TKN
July 13	7.15	7.21	4.20	4.35	-	-0.8*	41.2	39.2	-
July 24	7.76	6.98	5.46	5.59	-	10.1	29.6	28.0	-
Aug. 5	7.62	7.22	6.23	5.74	-	5.3	18.2	24.7	-
Aug. 23	7.89	8.55	4.71	5.89	-	-8.4*	40.3	25.4	-
Sept. 1	7.69	7.46	5.74	7.11	-	3.0	25.3	7.5*	-
Oct. 22	7.71	6.99	3.83	4.98	5.36	9.3	50.3	35.4	30.5
Oct. 31	7.63	7.86	3.58	4.95	5.45	-3.0*	51.1	35.1	28.6
Nov. 10	7.65	8.95	6.99	4.86	8.24*	-17.0*	8.6	36.5	-7.7*
Nov. 23	7.36	6.74	4.31	4.26	5.21	8.4	41.4	42.1	29.2
Dec. 4	7.53	7.47	4.09	4.48	5.36	0.8	45.7	40.5	28.8
Mean	7.60	7.54	4.91	5.22	5.35	6.1	35.2	34.1	29.3
Standard error of estimate of mean	0.07	0.23	0.36	0.28	0.05	1.5	4.5	2.2	0.4

* rejected, due to suspected contamination

not change by more than 0.2 units for all samples collected up until mid October. The pre-concentration pH values of samples collected in the late fall and early winter were slightly lower with a mean of 7.6 versus 8.2 for the summer and early fall samples. Upon concentration, the lower pH values climbed to a mean of 8.1.

Electrolytic Respirometer Experiments

During the course of the concentrated sample runs an inorganic precipitate formed. The mass of this precipitate was measured gravimetrically. This mass compared very well with the loss of alkalinity as calcium carbonate and is shown in Table IV-5. During a run the precipitate settled on the seed cores thus potentially smothering the organisms and also making it virtually impossible to obtain an accurate measure of biomass. Therefore, it was necessary to assume that all 4 reactors contained the same amount of biomass. To avoid these problems, a primary effluent was adopted as a seed to replace the seed cores.

The results from the series of oxygen demand experiments at 20°C on concentrated samples are shown in Table IV-6 (the data and oxygen demand plots for each run are shown in the Appendix, Tables A1-A7 and Figures A1-A7). The mean and standard error of estimate for the mean of the actual recorded values are provided. As well, these statistics are provided for each parameter after being normalized back to the original sample concentration on the

TABLE IV-3. Effect of concentration on nitrite and nitrate.

Sample Date	Before Concentration*		After Concentration*	
	NO_2^- µg/l as N	NO_3^- µg/l as N	NO_2^- µg/l as N	NO_3^- µg/l as N
Oct. 31	0.5	8.6	0.0	28.3
Nov. 10	2.0	1.9	0.0	10.5
Nov. 23	2.2	24.9	0.0	1.0
Dec. 14	2.0	74.0	0.0	2.1

* sample concentration factors are given in Table IV-2

TABLE IV-4. Effect of concentration on pH.

Sample Date	pH before concentration	pH after concentration
before ice formation		
July 13	8.3	8.2
July 24	8.4	8.3
Aug. 3	8.3	8.2
Aug. 23	8.2	8.0
Sept. 1	8.3	8.2
Mean	8.3	8.2
during ice formation		
Oct. 22	7.4	8.0
Oct. 31	7.8	8.1
Nov. 10	7.8	8.2
Nov. 23	7.5	8.1
Dec. 4	7.7	8.3
Mean	7.6	8.1

TABLE IV-5. Alkalinity loss and the mass of inorganic precipitate at the end of the electrolytic respirometer runs.

Sample Date	Reactor #	Inorganic ppt. mg/l	Alkalinity loss mg/l as $CaCO_3$
July 13	1	206	194
	2	276	186
	3	362	232
July 24	2	227	382
	3	290	274
Aug. 5	1	292	320
	2	282	306
	3	356	386
Aug. 23	1	238	250
	2	244	264
	3	260	264
Sept. 1	1	144	178
	2	199	212
	3	234	268
Mean		258	265
Standard error of mean estimate		15.7	17.6

basis of each mass balance concentration factor (from Table IV-2). In the case of BOD, the volume reduction concentration factor (which is almost equivalent to the TOC concentration factor) was used for normalization. The nitrite and nitrate normalization was done on the basis of the TKN concentration factor (this is based on the assumption that TKN loss is converted to nitrite and nitrate). The net IC loss was calculated by the difference of IC loss, measured by combustion, and the alkalinity loss as inorganic carbon (which is roughly equivalent to the carbon mass in the precipitate). IC loss was quite high and consistent when the primary effluent seed was used. However, when seed cores were used the IC loss was extremely inconsistent, in fact in a few instances an IC gain was observed. This was possibly due to soluble inorganic carbon from the cores so these values were not included in Table IV-6. Two TOC results were also excluded from Table IV-6 since a net increase of TOC was observed in these cases despite significant oxygen demand exerted. The net increase in TOC is possibly due to contamination or analytical errors and since no plausible alternate explanation could be determined these results were rejected. The nitrite and nitrate creation levels represent the maximum values observed. The largest increase in nitrite and nitrate was observed midway through the run. The levels of nitrite and nitrate then decreased by an average of 79.1 µg/l-N and 33.6 µg/l-N respectively by the end of the run, thus suggesting a

TABLE IV-6. Oxygen demand and substrate conversion for electrolytic respirometer experiments at 20°C on concentrated samples.

Sample Date	Reactor #	Seed	Measured BOD mg/l	TOC removal mg/l	IC removal mg/l	TKN removal mg/l as N	max. NO_2^- creation µg/l as N	max. NO_3^- creation µg/l as N
July 13	1	1°effl.*	20.8	10.0	53.3	-	-	-
	2		32.0	10.6	58.5	-	-	-
	3		30.0	13.9	55.5	-	-	-
July 24	2	cores	24.0	4.0	(-12.8)	-	-	-
	3		25.5	6.0	(16.1)	-	-	-
Aug. 5	1	1°effl.*	24.0	-	66.2	-	-	-
	2		13.3	-	39.9	-	-	-
Aug. 23	1	cores	17.4	10.2	(15.0)	-	-	-
	2		20.4	18.4	(-6.4)	-	-	-
	3		50.4	17.8	(47.6)	-	-	-
Sept. 1	1	cores	27.6	6.2	(13.6)	-	-	-
	2		13.3	0.0	(19.6)	-	-	-
	3		-	4.3	(48.3)	-	-	-
Oct. 31	1	cores	29.0	2.2	(-25.0)	1.28	-	-
	2		9.4	3.6	(-0.9)	0.15	-	-
	3		3.4	4.0	(21.9)	-	-	-
Nov. 10	1	1°effl.*	29.0	12.2	73.6	0.93	82.0	29.9
	2		10.0	10.4	74.5	0.85	82.0	32.1
	3		38.0	11.0	73.5	0.22	83.0	27.8
Mean Value			23.2	8.5	61.9	0.69	82.3	29.9
Standard error of mean estimate			2.7	1.3	4.4	0.2	0.3	1.2
Mean value of all values normalized to orig. conc.			3.0	1.1	11.0	0.13	16.9	6.2
Standard error of mean estimate			0.4	0.2	0.8	0.04	0.07	0.3

* primary effluent

sink for nitrite and nitrate during the run. Results for nitrite and nitrate were monitored only at the end of the run for the October 31 samples and showed no change in nitrite and a net decrease in nitrate. These results were excluded from Table IV-6.

The two sets of data obtained using seed cores and primary effluent seed were combined in Table IV-6. A "t" test (Guttman et al, 1971) indicated that neither of the mean values for BOD and TOC for the two methods of seeding were significantly different at the 5% significance level. Similarily, the "t" test indicated that neither of the mean values for BOD and TOC for each reactor in each run were significantly different at the 10% significarce level.

A typical set of oxygen demand curves at 20°C for 3 reactors using a concentrated sample and seeded blank are shown in Figure IV-3.

Figures IV-4 and IV-5 show the results of an analysis done on two runs (July 13 and November 10) to assess how well the kinetic course of the BOD reaction conforms with first order reaction kinetics, which is expressed as (Metcalf and Eddy, 1979):

$$\frac{\partial L_t}{\partial t} = -kL_t$$

where L_t is the amount of BOD remaining at time, t. The equation can be integrated as:

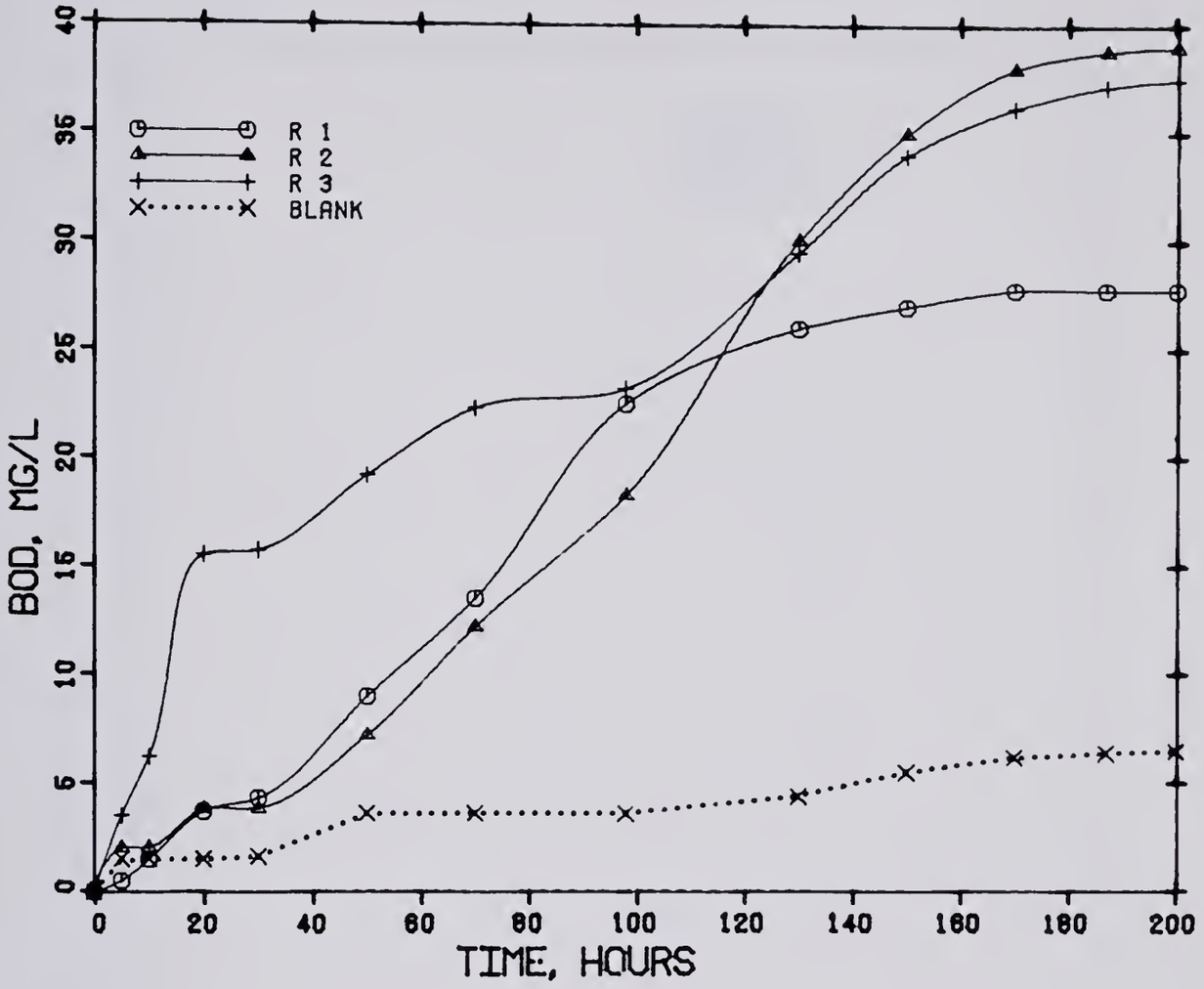


FIGURE IV-3. Typical oxygen demand progression at 20°C on concentrated river water (July 13, 1978).

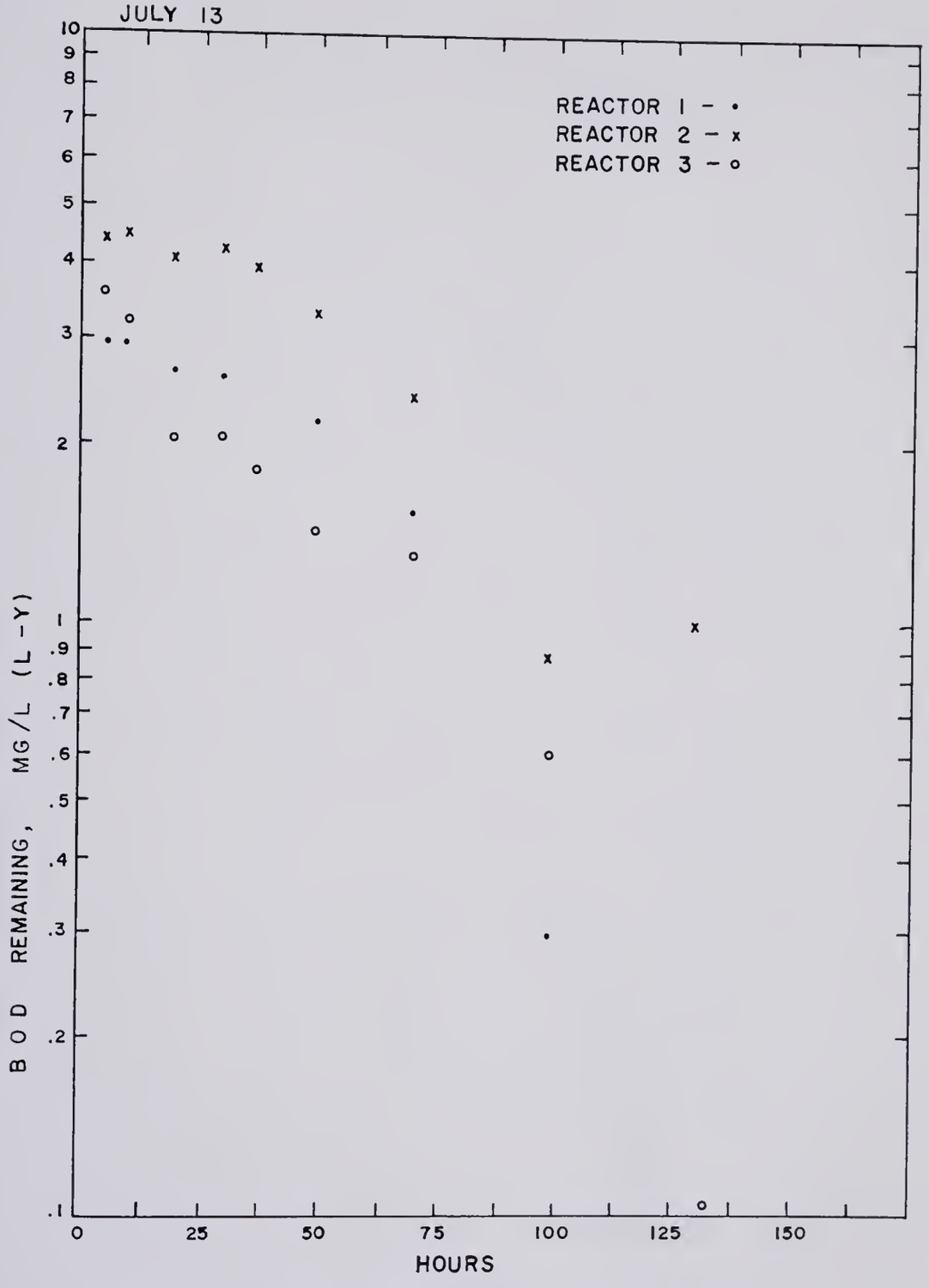


FIGURE IV-4. Biochemical oxygen demand remaining versus time: a test for 1st order kinetics of the rate of oxygen uptake in a 20°C concentrated sample electrolytic respirometer run, July 13, 1978.

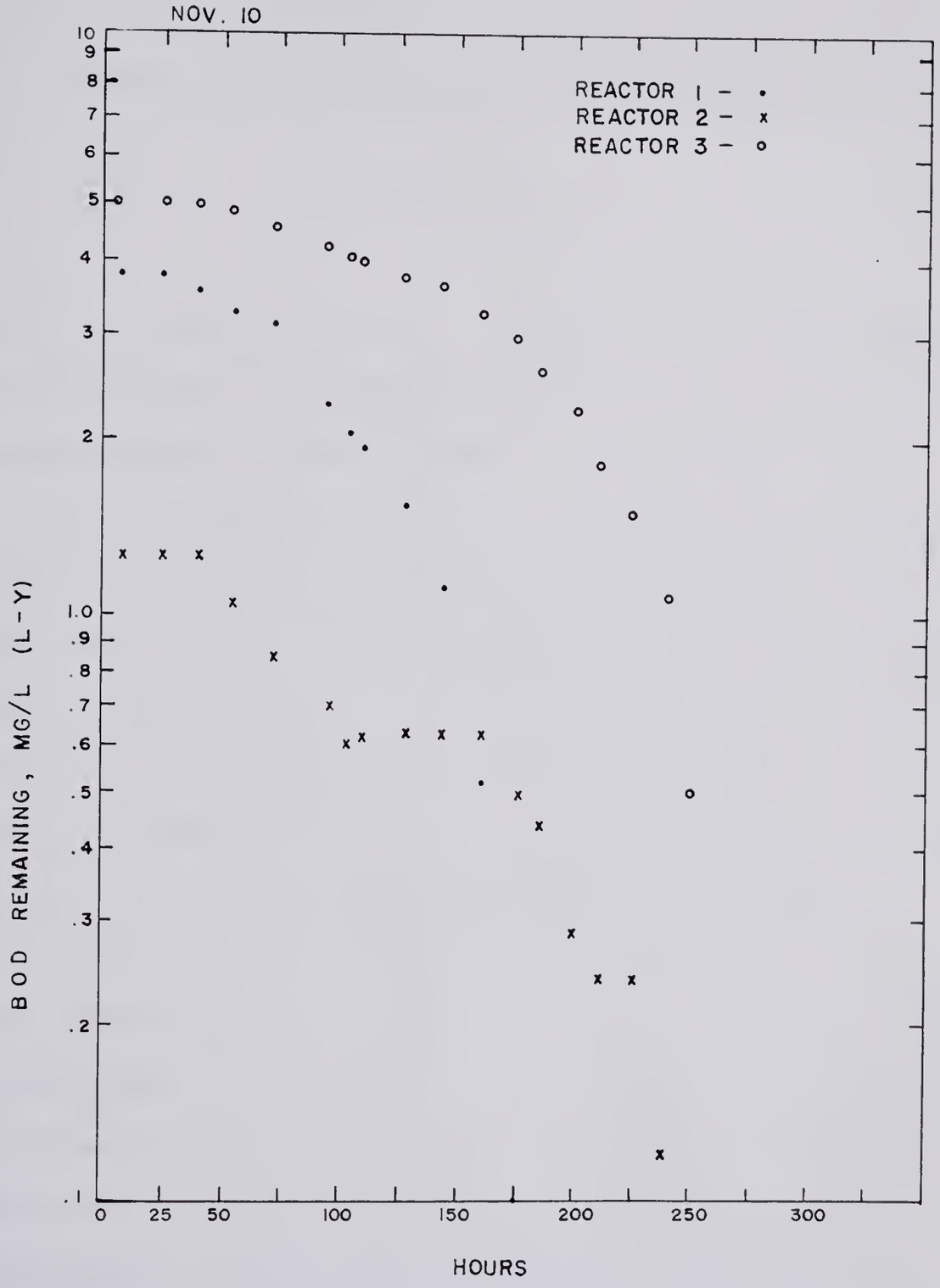


FIGURE IV-5. Biochemical oxygen demand remaining versus time: a test for 1st order kinetics of the rate of oxygen uptake in a 20°C concentrated sample electrolytic respirometer run, November 10, 1978.

$$\log L_t \Big|_0^t = -Kt$$

$$\log L_t = \log L - Kt$$

where L is the amount of BCD at t=0 (ultimate BCD).

Figures IV-4 and IV-5 are plots of log L_t vs t where

$$L_t = L - y$$

L is the normallized (with respect to the liquid volume reduction) BOD value at the end of the run and y is the normalized BCD value at time t.

The raw data and oxygen demand plot for the 20°C run using a concentrated sample with 5 mg/l of 1-Allyl-2Thiourea (ATU) (a nitrifying inhibitor) are shown in the Appendix, Table A8 and Figure A8. The mean measured BOD (using seed cores) was 10.3 mg/l (23.0, 8.8 and 1.2 mg/l for reactors 1, 2 and 3 respectively) with a standard error of mean estimate of 6.7, or 1.3 mg/l and 0.9, respectively, after normalization. The observed TOC removal was 1.1 mg/l for both reactors 1 and 2 (taking into account the 2.1 mg/l of carbon added in the form of ATU). Reactor 3 indicated a net increase in TCC. In terms of the original concentration, the TOC removal was 0.2 mg/l for reactors 1 and 2. No precipitate was observed in this run. Net gains were observed in IC and TKN (despite taking into account the 1.2 mg/l-N added in the form of ATU). A net loss was observed in

nitrate (which was measured only at the end of the run) and nitrite remained unchanged except in reactor 1, which showed an increase of 127 µg/l-N (or 23.7 µg/l-N in terms of the original concentration).

The unconcentrated, 20°C sample run (Appendix A, Table A9 and Figure A9) had observed BOD's of 20.2 mg/l for reactor 1 and 0.8 mg/l for reactor 2 (reactor 3 malfunctioned). The corresponding TOC removals were -0.3, 0.3 and 1.5 mg/l for reactors 1, 2 and 3. No precipitate was observed in this run and the observed IC loss was 5.9 mg/l. Unfortunately, a nitrogen balance was not done on this sample run.

The results and statistical analyses from the series of oxygen demand runs at 4°C are shown in Table IV-7 (the raw data and oxygen demand plots are shown in the Appendix, Tables A10 and A11 and Figures A10 and A11). As in the 20°C runs, statistics are provided for each parameter after being normalized back to the original sample concentration on the basis of each mass balance concentration factor (from Table IV-2). All test results obtained at 4°C used the primary effluent seed. In the case of the November 23 sample, nitrite concentration reached a meximum midway through the run and decreased towards the end of the run. Nitrate concentration values were roughly equivalent midway through the run and at the end of the run. For the December 14 sample, nitrogen analysis was done only at the beginning and end of the run.

A typical set of oxygen demand curves at 4°C for 3 reactors using concentrated sample and seeded blank are shown in Figure IV-6.

The unconcentrated, 4°C sample run (Appendix, Table A12 and Figure A12) showed very erratic oxygen exertion; 48.2 mg/l for reactor 1; 8.4 mg/l for reactor 2 and; 0 mg/l for reactor 3. The average TOC's were 0.2, 0.3 and 0.6 mg/l for reactors 1, 2 and 3 respectively. The average IC loss for the 3 reactors was 18.4 mg/l. Nitrogen analysis showed an average TKN loss of 0.1 mg/l-N, a nitrite creation of 23 µg/l-N in reactor 1 and 0 µg/l-N in reactors 2 and 3, and showed an average decrease in nitrate of 11.9 µg/l-N. Nitrogen analysis was performed only at the begining and end of the run.

Analysis of the blanks (control samples), before and after each run, indicated a negligable change in TOC from the start to the finish of a run. The observed levels of organic carbon in the blanks (about 2 mg/l) approached the sensitivity of the carbon analyzer.

HPLC Analysis

The chromatograms obtained from this analysis represent a measure of only those components in solution that can absorb light at a wavelength of 254nm. Therefore, it is not a measure of the total organics present.

Chromatograms for the fulvic acid standard and a concentrated sample are shown in Figures IV-7 and IV-8.

TABLE IV-7. Oxygen demand and substrate conversion for electrolytic respirometer experiments at 4°C on concentrated samples.

Sample Date	Reactor #	Seed	Measured BOD mg/l	TOC removal mg/l	IC removal mg/l	TKN removal mg/l as N	max. NO_2^- creation µg/l as N	max. NO_3^- creation µg/l as N
Nov. 23	1	1°effl.*	10.4	3.6	57.3	0	9	77.6
	2		3.9	2.2	54.9	0	4	88.0
	3		2.7	0.6	63.5	0	6	71.0
Dec. 14	1	1°effl.*	13.0	1.0	25.9	0.58	0	416.3
	2		9.2	6.0	26.9	0.80	1	415.3
	3		12.0	-	29.0	0.44	0	426.5
Mean Value			8.5	2.7	42.9	0.30	3.3	249.1
Standard error of mean estimate			1.8	1.0	7.1	0.1	1.5	76.2
Mean value of all values normalized to orig. conc.			1.1	0.4	10.1	0.1	0.6	46.5
Standard error of mean estimate			0.2	0.1	1.6	0.0	0.3	14.2

* primary effluent

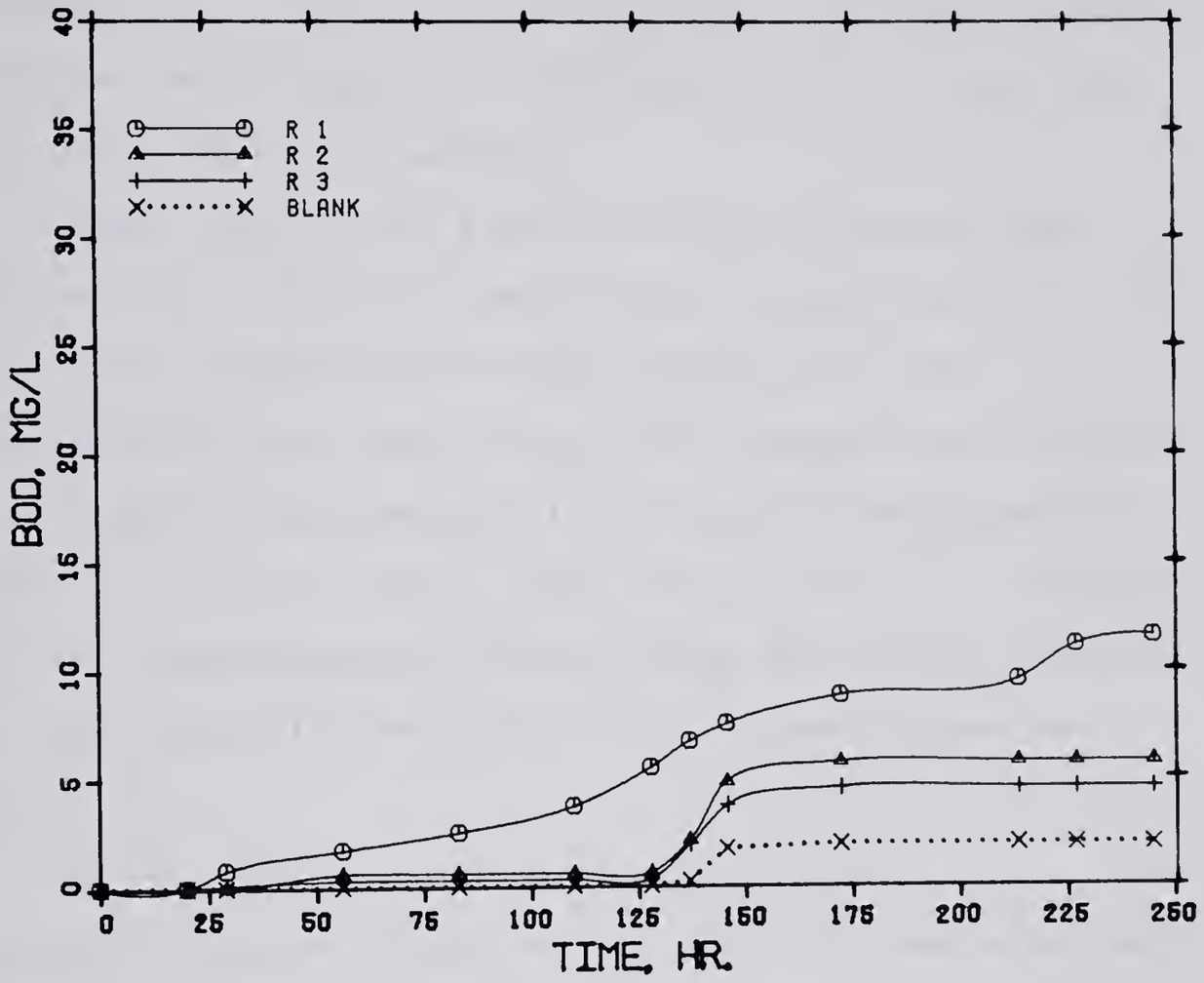


FIGURE IV-6. Typical oxygen demand progression at 4°C on concentrated river water (November 23, 1978).

These chromatograms were run using a linear solvent gradient of 100% acetonitrile to 100% water over 20 minutes. Both standard and sample were run at a pH of 2. Due to the difficulty in maintaining pressure and a consistent solvent gradient, reproducibility of the chromatograms were poor from one injection to another.

Figure IV-9, gives examples of the chromatograms obtained for a distilled water blank, unconcentrated river water, concentrated river water before a run and concentrated river water after a run. These chromatograms were obtained using using 1:1 acetonitrile/water solvent system and all the samples were run at a pH of 2. Compared with the chromatograms obtained using the solvent gradient, the reproducibility of each of these chromatograms was excellent.

The chromatograms obtained using the 1:1 acetonitrile/water solvent system indicated two major peaks. These peaks had retention times of 3 and 4 minutes. The two peaks were evident in all samples except for the blank which only had the one peak at 4 minutes. A comparison of the peak areas of the first peak in each sample are shown in Table IV-8. The peak areas were determined using the height-width (width at 1/4 height) triangulation method and are expressed in terms of the original concentration based on the TOC concentration factor.

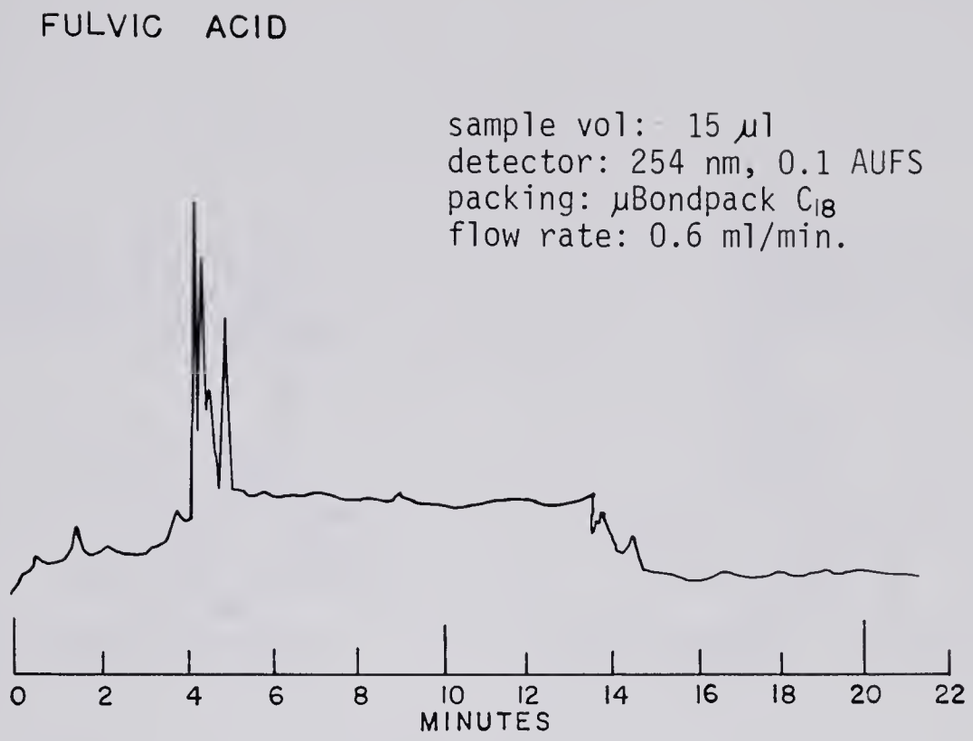


FIGURE IV-7. A linear solvent gradient chromatogram of a fulvic acid standard using HPLC. (solvent gradient: 100% acetonitrile to 100% water over 20 min.)

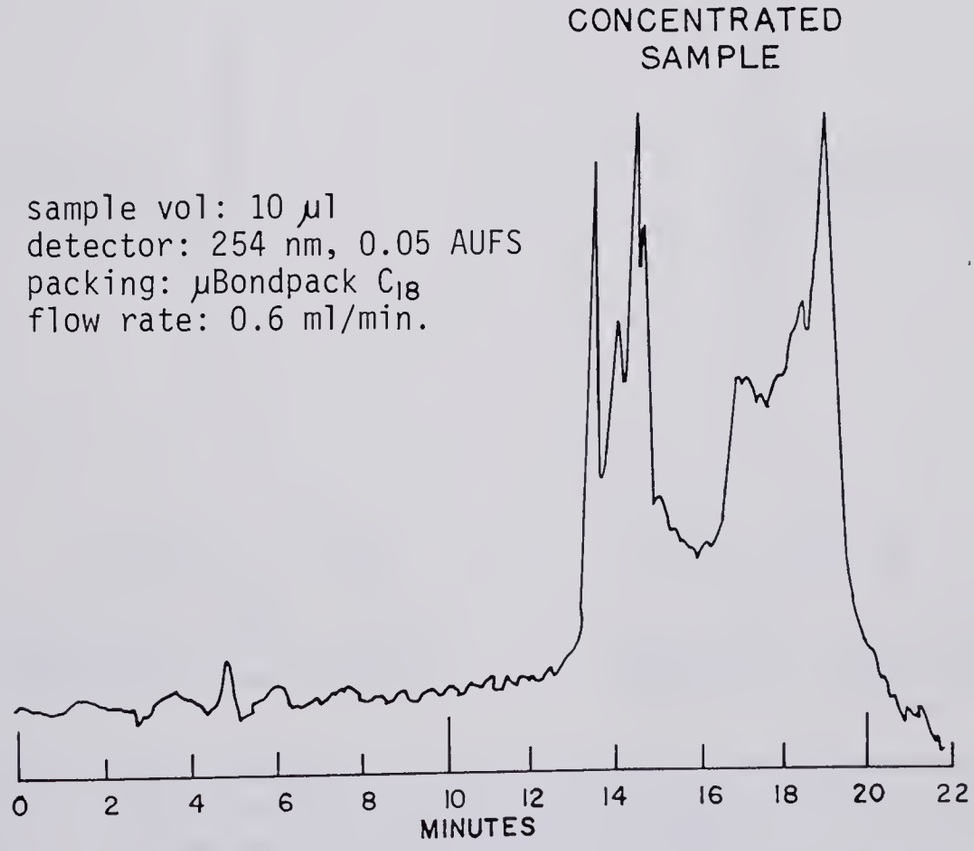
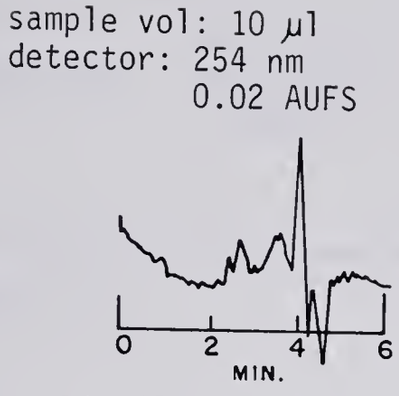
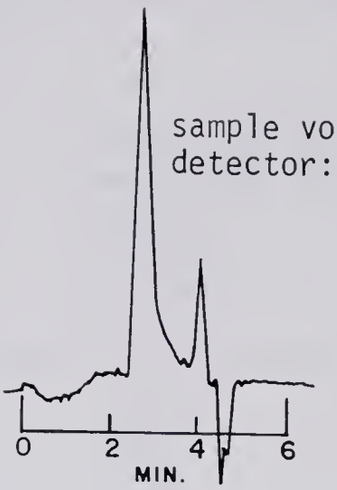


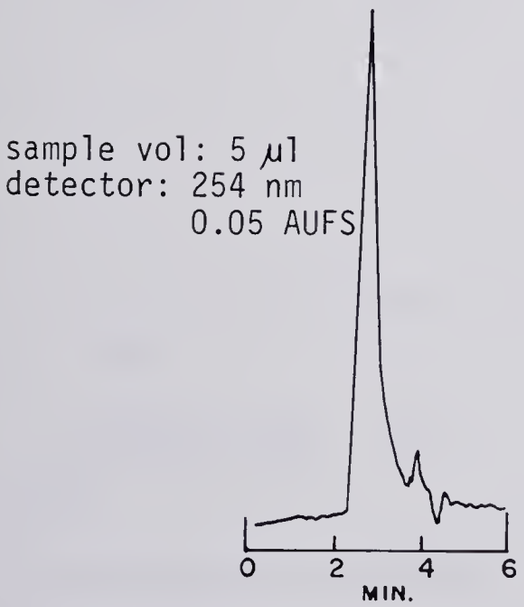
FIGURE IV-8. A linear solvent gradient chromatogram of concentrated river sample using HPLC. (solvent gradient: 100% acetonitrile to 100% water over 20 min.)



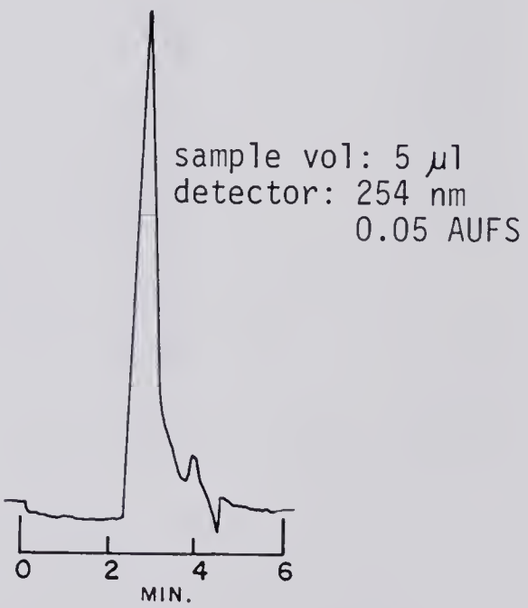
Distilled Water



River Sample, original conc.



Concentrated river sample
-before E.R. experiment-



Concentrated river sample
-after E.R. experiment-

FIGURE IV-9. Chromatograms of distilled water, unconcentrated river sample and concentrated river samples before and after an electrolytic respirometer (E.R.) experiment. (solvent: 1:1 acetonitrile/water, packing: µBondpack C_{18}, flow rate: 0.6 ml/min.)

TABLE IV-8. Peak areas, normalized to the original concentration and adjusted to an equivalent injection volume and detector absorbance range.

Sample Date	Peak areas of unconc. samples	TOC conc. factor	Normalized and adjusted peak areas of concentrated samples: Before E.R. run*	After E.R. run*
July 13	-	7.03	1.03	1.03
		7.03	0.99	0.96
		7.65	1.10	1.36
		7.65	1.18	1.29
Aug. 5	1.16	7.38	0.93	1.04
	0.99	7.38	1.05	1.07
		6.65	0.99	1.10
		6.65	1.06	0.95
		7.62	0.97	1.02
		7.62	1.05	1.01
Aug. 23	0.90	9.07	1.03	0.84
	1.01	9.07	1.04	0.78
		9.07	1.05	0.99
		9.07	1.04	1.00
		8.26	0.73	0.81
		8.26	0.75	0.85
		8.34	0.81	0.87
		8.34	0.78	0.87
Mean	1.01	-	0.98	0.99
Standard error of estimate of mean	0.05	-	0.03	0.04

* electrolytic respirometer experiment

V. DISCUSSION

Physical and Chemical Properties of the Blindman River

The decrease in flow of the Blindman River during the winter months is attributed to two major factors: (1) the decrease in ground water flow due to a decrease in infiltration and; (2) the involvement of a large amount of river water in ice formation. Because ice formation involves essentially only pure water and low ground water flow suggests decreased dilution, these factors can be considered as the cause of the increased levels of alkalinity and IC during the period of ice formation. From November 10 to January 4 alkalinity and IC approximately doubled in concentration. This is consistent with the observed liquid water depth decrease by approximately the same factor (from 1-1.5 m to 0.4-0.9 m) during the same period. The slight decrease in pH during ice cover is probably attributed to the increase in bicarbonate concentrations (which is roughly equal to the alkalinity and IC levels expressed as bicarbonate).

Despite the changes in flow, alkalinity and pH from July to January, the organic carbon and organic nitrogen (as represented by TKN since ammonia concentrations were negligible) concentrations were relatively constant. The organic nitrogen to organic carbon ratio observed (about 6%) is consistent with that expected for humic substances. Figures reported by Gjessing (1976) on the major elemental content of humic substances (43-63% carbon and 0.5-5%

nitrogen) indicate that the nitrogen to carbon ratio would be 0.8 to 11%. However, Gjessing pointed out that, based on his experience in Norway, aquatic humus has lower carbon and nitrogen levels as compared with soil humus and exhibit a nitrogen to carbon ratio of about 3%. The value obtained for the Blindman River was above this figure but well within the overall range. For the sake of comparison, the nitrogen to carbon ratio for protein is on the average 29%, well above the value obtained for this investigation.

The observed increase in nitrate from October 31 to January 4 cannot be assessed without further field investigations.

The low level of dissolved oxygen observed on January 4 (4.1 mg/l) is typical of streams in this area during this time of year. Such low levels may have serious implications on the process of self-purification further downstream.

The observed decrease in TKN and increase in nitrate in the 6.4 km stretch between the site at Blindman River near Blackfalds and the site near the mouth of the river, suggests the occurrence of nitrification. Also, the slight decrease in BOD and TOC along this stretch suggests that biological utilization of organic carbon is occurring. However, it should be realized that the BOD and TOC values are approaching the sensitivity of the analytical methods. The degree to which nitrification and carbonaceous biological oxidation is occurring along this stretch cannot be assessed without a more thorough investigation. Factors

such as, the fate of the parameters (in the water column) being investigated, ground water (which increases the river flow, dilution and the chemical content) and others should all be taken into account.

The increase in dissolved oxygen in this 6.4 km stretch is attributed to a few sections of open water along this stretch. This agrees with Landine's (1970) observation that any opportunity for reaeration is extremely beneficial in the oxygen economy in streams under winter ice conditions.

The field investigations, though minimal, were considered valuable in order to obtain a feel for the behaviour and complexity of the true system.

Concentration Procedure

The study conducted on the effect of the concentation procedure on BOD indicated that the normalized BODs for concentrated samples were lower than the BODs for the unconcentrated sample (Figure IV-1). This may be attributed to either the loss of readily degradable and/or volatile organics in the concentration step or the development of inhibitory conditions at higher concentrations (particularily the increase of inorganic ions, principally bicarbonate). The relatively small average loss of organic carbon (6.1%), in the concentration process, as indicated in Table IV-2, tends to suggest that the second hypothesis may be most important in the case of the sample concentrated by a factor of 2 (in which no precipitate occurred). However,

this observed loss of TOC may significantly account for the case of the normalized BOD concentrations of the more highly concentrated samples. At the higher concentration levels, where precipitation of carbonate occurred, the effect of inhibition and/or the effect of the loss of TOC was judged to be acceptable for this study.

The evaluation of the effect of concentration on the rate of DO uptake by semi-continuously monitoring the DO in a standard bottle BOD test (Figure IV-2), indicated a decrease in lag time and an increase in the rate of oxygen uptake. The decrease in lag time may be due to an increase in the potential for organism to substrate contact in the concentrated sample. However, assumptions were made with respect to the second observation. It was assumed that the two stages observed in the unconcentrated sample were the carbonaceous and nitrogenous stages respectively, and that the nitrogenous stage was not yet reached in the concentrated sample in this time period. If this is the case, then it can be concluded that the carbonaceous oxygen demand rate is much more rapid in the concentrated sample (by about a factor of 3) than in the unconcentrated sample (thus suggesting that the rate is dependent upon concentration). However, without performing a nitrogen balance on these samples or controlling nitrification, one cannot be sure if the carbonaceous and nitrogenous oxygen demands are occurring concurrently or if the nitrogenous oxygen demand is occurring at all. Therefore, the result of

this test with respect to the rate of oxygen uptake is inconclusive.

It should be noted, that the BOD at the end of 4.5 days (108 hours) of the unconcentrated samples in Figures IV-1 and IV-2 were 1.5 and 8 mg/l, respectively. This difference is due to the sensitivity of this BOD test to mixing. The samples monitored continuously required vigorous mixing at the time of each DO measurement in order to obtain a reliable result.

The concentration factor and component loss data in Table IV-2 indicated good agreement between the losses of IC and alkalinity. These losses are attributed to the precipitation of calcium carbonate during the concentration process. Significant losses also occurred in TKN, which is probably due to the adsorption of the nitrogenous organic compounds onto the inorganic precipitate and glassware. The loss of TOC in the concentration process was minimal and may be attributed to the loss of volatile organic compounds and/or adsorption of some compounds onto the precipitate and glassware. The increase in TOC, observed in some cases, were attributed to contamination of the glassware and/or absorption of volatile organics from the laboratory environment while preparing the sample for analysis and most likely due to the difficulty in obtaining reagent grade water, having a TOC below 2 mg/l, for dilution. The increase in pH of the winter and late fall samples upon concentration was probably due to the loss of carbon dioxide. The fate of

nitrite and nitrate in the concentration process is an unanswered question. However, the possibilities appear to be denitrification and/or some form of chemical binding by the aquatic humic substances.

The concentration procedure was a very important aspect in this investigation, since it was necessary to obtain measurable parameter changes using the equipment available. It was found that sample concentration was acceptable for this study. The justification for relating each major parameter in the concentrated sample back to the original sample concentration was based on the test conducted on BOD as a function of concentration.

Electrolytic Respirometer Experiments

The stated objectives of the experimental program were to attempt to determine if significant biochemical oxygen demand could be demonstrated on the natural organic matter in the river system and if found could it be demonstrated at low temperature conditions.

The first objective was achieved as indicated in Table IV-6 and Figure IV-3. The data indicated a mean, normalized, plateau oxygen demand value of 3.0 ± 0.4 mg/l at 20°C. These oxygen demand values were possibly under-estimated since no correction was taken into account for the apparent inhibition and/or the loss of potentially biodegradable organics during the concentration process. The oxygen demand time progression was consistent with that expected of a

substrate limited biochemical oxygen demand experiment. The corresponding organic carbon removal for the BOD exerted was 1.1 ± 0.2 mg/l. Since the degree of oxidation and relative biodegradability of the organic matter in the sample was unknown, it was not possible to propose a stoichiometry for the mass of biochemical oxygen demand per unit mass of organic carbon. However, by comparing the BOD/TOC ratio (based on the original TOC present in solution) of untreated domestic sewage, about 1.4 (Metcalf and Eddy, 1979), and the concentrated river samples, about 0.2 (TOC values obtained from Appendix A, Tables A1-A7), gives an indication of the resistance of the organic carbon present in the river samples to biochemical oxidation.

By comparing the ratio of observed oxygen demand to TOC loss in the sample with the theoretical ratios of certain organic compounds, another perspective on the nature of the organic matter present in the sample can be obtained. Since all organic carbon analysis was performed on membrane filtered samples, organic carbon converted to new cell mass (along with the evolution of carbon dioxide) is considered as organic carbon loss. For the concentrated river samples, the mean ratio of BOD to TOC removal was 2.7. This may be compared with the theoretical total oxygen demand to organic carbon ratios (assuming complete oxidation to carbon dioxide and water) ranging from 2.67 for glucose, 4.66 for ethanol and 5.33 for methane. The actual ratios for these pure compounds would be lower due to the diversion of some

organic matter to new cell mass. This then suggests that the organic matter responsible for BOD in the river water samples may have been present in a partially oxidized state rather than being highly reduced.

Also, by comparing the shapes of the oxygen demand curves obtained for a river sample, Figure IV-3, with that of a typical electrolytic respirometer run using glucose--glutamic acid (a "simple" BOD reaction), Figure V-1, it may suggest that the compounds responsible for the oxygen demand in the river samples are being generated during the course of the run from the breakdown of larger, more resistant compounds. This may also give an indication of the concurrent occurrence of nitrogenous and carbonacous oxygen demands.

At the end of a run there was an observed IC loss (when the primary effluent was used) after taking into account the precipitate mass (Table IV-6). This loss can be attributed in part to carbon dioxide loss in the system however, this would be very minor. Another possibility to account for the loss would be through the utilization of inorganic carbon as a carbon source by autotrophic bacteria during the process of nitrification. The occurrence of nitrification was observed (Table IV-6) by the decrease in TKN and increase in nitrite and nitrate. The actual nitrogenous oxygen demand (NOD) contribution can be estimated using the relationship proposed by Wezernak and Gannon (1968), equation II-8.

The estimated NOD from the data collected for a

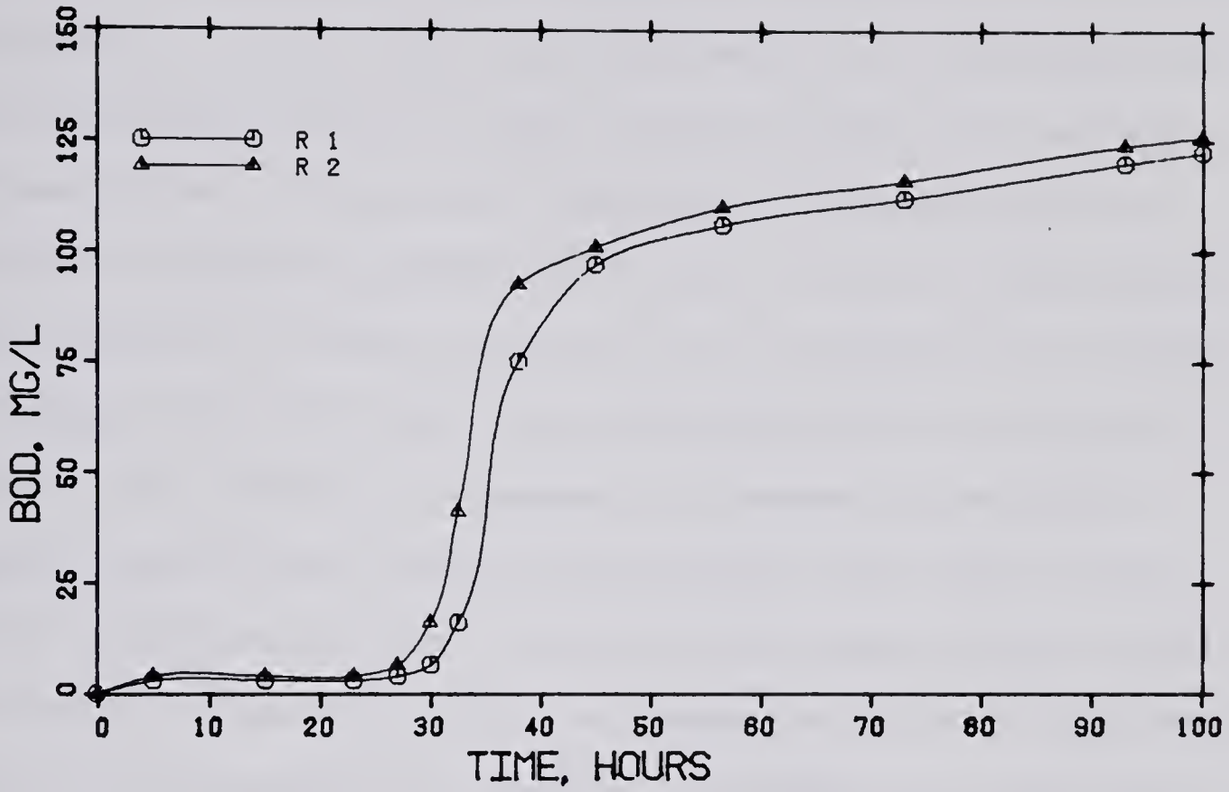


FIGURE V-1. BOD "reaction" curves of glucose/glutamic acid (150 mg/l) using the electrolytic respirometer. (20°C)

nitrogen balance in Table IV-6 is based on the assumption that the TKN loss during the run is all converted to nitrate except for the measured increase in nitrite. (The measured nitrite is subtracted from the nitrite to nitrate conversion term). The validity of this assumption is dependent upon the possibility of TKN loss to precipitation and assimilation, since the measurement of final ammonia showed that no TKN had been left in this form. Therefore, on this basis the calculated normalized NOD is 0.5 mg/l or 17% of the observed oxygen demand, at 20°C. Theoretically, it should be possible to confirm this NOD value by performing a NOD calculation based on the observed increases in nitrate and nitrite. However, samples collected approximately half way through the runs exhibited higher nitrate values than those obtained at the end of the run. This then suggests a sink for nitrate and/or nitrite within the system. Therefore, by using the maximum observed values of nitrite and nitrate created during a run in the NOD calculation, it would not be representative of the total oxidative conversion of organic nitrogen to these inorganic compounds. The only way this could be done is by knowing the degree and rate at which these compounds are disappearing. There are two possibilities which may account for the disappearance of nitrate and/or nitrite. One possibility is denitrification which may occur within possible anaerobic microzones around the inorganic precipitate. Another possibility is that ammonia and nitrite may have been scavenged by the humic

substances in solution. The latter, is probably the most significant contributing factor to the decrease in these nitrogenous compounds since it has been readily observed and documented by several other investigators (Sorensen, 1962; Steelink, 1963; and Rashid and King, 1969).

There was a significant difference in the IC losses between the runs using a different method of seeding. This difference was probably due to the release of inorganic carbon from the seed cores during a run, thus increasing the IC levels in these runs. However, there was an insignificant difference (at the 5% level) between the TOC removal and BOD results using seed cores and the primary effluent seed, which suggests that the bacterial cultures in both cases behaved similarily in their response to the substrate. This observation is consistent with Gordon's (1970) hypothesis regarding proteolytic bacteria in the Chena River, Alaska, at low temperatures. He suggested that proteolysis was one of the major metabolic activities of the bacteria in the Chena River at low temperatures and he indicated that there were probably similar bacteria in sewage treatment systems operating at low temperatures.

Figures IV-4 and IV-5 indicated that the kinetic course of the BOD reaction of the concentrated river sample does not conform with first order kinetics (indicated by the non-linearity of the data points). First order kinetics has been used to describe "simple" BOD reactions such as, the glucose-glutamic acid system, shown in figure V-1. However,

these river samples do not represent "simple" BOD reactions, as demonstrated by the shape of the oxygen demand curves. The possible simultaneous occurrence of nitrogenous and carbonaceous oxidation and the possible sequential breakdown of humus resulting in the generation of more readily oxidizable organics during the run complicates the kinetics of the reaction and makes a kinetic study extremely difficult.

The results obtained from a run using a concentrated sample at 20°C with allyl thiourea (ATU), indicated a lower (by 56%) average oxygen demand than those samples without ATU. The scatter in the results were however, quite large, so the significance of the few results are questionable. The TOC loss in this run was also substantially lower (by 81%) than the other 20°C concentrated runs. No substantive information regarding nitrification could be obtained from the erratic data. The increase in TKN suggests a possible analytical error and therefore, offers no clue to the occurrence of nitrification. However, if the decrease in oxygen demand was due entirely to the inhibition of nitrification the loss of TOC would be consistent with the other 20°C runs, which was not the case. The fact that no inorganic precipitate occurred in this run, possibly due to the cation complexation capability of ATU (principally with calcium) may account, in part, for the low BOD and TOC removal by maintaining inhibitory high concentrations of inorganic material. The observed increase in IC is not

surprising since seed cores were used in this run. Considering the observed inconsistency of the results in this run, several more samples would have to be run in order to obtain more conclusive results.

As expected, the unconcentrated 20°C sample run exhibited erratic TOC removal and BOD results. This is consistent with Gjessing's (1976) observations with respect to BOD measurements on humic substances. During the course of this run, no inorganic precipitate occurred due to the relatively low IC levels in solution. However, the observed net loss of IC was probably due to the loss of carbon dioxide and through utilization by autotrophic bacteria. The results obtained from this run tend to support the fact that the parameters of interest must be present in larger quantities in order for them to be reliably monitored.

The second objective of this investigation was satisfied with the data provided in Table IV-7 and Figure IV-4. These data indicate that a measurable biochemical oxygen demand could be measured for the natural organic matter at a temperature approaching the winter, under ice, condition.

The 4°C concentrated sample experiments indicated an apparent plateau BOD of 1.1 ± 0.2 mg/l (normalized mean) with a corresponding organic carbon removal of 0.4 ± 0.1 mg/l (normalized mean). Both, the oxygen demand exerted and the TOC removed, at this temperature, was about 63% lower than that observed at 20°C. This is attributed to a decrease

in the metabolic rates of microorganisms.

The NOD estimated for the low temperature experiments, using the same assumptions as used for the 20°C experiments indicated a value of 0.4 mg/l or 36% of the observed oxygen demand. This indicates that the relative importance of the estimated NOD at 4°C for explaining the observed oxygen demand is considerably greater than at 20°C. Once again, the degree of nitrification occurring based on TKN loss may be questionable since this loss may be due to precipitation and/or assimilation. However, the occurrence of nitrification can be confirmed by the observed increase in nitrate. Similarily, the IC loss can be attributed, in part, to the process of nitrification through IC utilization by autotrophic bacteria. Although based on a small amount of data, the findings suggest that the nitrogenous oxygen demand should not be overlooked in evaluating the winter dissolved oxygen depletion problem.

The unconcentrated 4°C sample run indicated erratic oxygen demand results, however, the TOC removal was consistent with the 4°C concentrated runs. The TKN removal was also consistent with the 4°C concentrated runs despite the fact that no precipitation of carbonate occurred. This may suggest that TKN removal through precipitation may be minimal. The occurrence of nitrification was also indicated in this run by the loss of TKN, increase in nitrite and loss of inorganic carbon.

HPLC Analysis

The HPLC analysis was performed on clear, acidified samples which, with respect to humus, would contain primarily the fulvic acid fraction. Since, according to Baker *et al* (1975), the total humic and fulvic acid content of the Red Deer River basin is 90% fulvic acid, it was most reasonable to examine this fraction.

Figures IV-7 and IV-8 indicated that, under similar conditions, there was no relationship between the standard solution and the concentrated sample. This would suggest that a standard solution should have been prepared from the humic substance found in the geographic area in which the investigation was taking place. The chromatograms do, however, indicate the complexity of the molecule(s) that was (were) being dealt with.

The chromatograms obtained using 1:1 acetonitrile-water indicated no degradation of the UV absorbing material through the course of a run, or in the concentration process, since chromatograms for all three types of samples had similar peak areas (after normalization). Since there was an observed organic carbon loss in each of the runs, this test suggests that the material which had undergone degradation did not involve the components which absorb at 254nm.

The consistency between the peak areas of the unconcentrated sample and corresponding normalized concentrated sample indicated that minimal loss of this UV

absorbing component occurred during the concentration process. This is consistent with the minimal loss of TOC observed in the concentration process.

Without a reliable standard and further chemical analysis, the UV absorbing component cannot be determined. However, the utilization of HPLC appears to have potential in characterization of such complex systems as aquatic humus. This method of analysis for humic substances would be best utilized in conjunction with other methods, unless a suitable standard can be obtained.

VI. SUMMARY AND CONCLUSIONS

Significant biochemical oxygen demand of concentrated pristine river samples, from the Blindman River, were demonstrated at both 20°C and 4°C in an electrolytic respirometer and is attributed to natural organic runoff. This oxygen demand could be related back to the original river water concentration. A carbon balance performed on these samples indicated that carbon losses (through metabolic activity) were lower at 4°C than at 20°C. A nitrogen balance indicated the occurrence of nitrification and the possibilities of denitrification, and chemical binding of some nitrogenous components by the aquatic humic substances at both temperatures. However, the relative importance of nitrification to the oxygen demand was greater at 4°C than at 20°C.

The way in which high pressure liquid chromatography (HPLC) was used in this investigation gave very little information regarding the degradation of natural organic substances in water. However, from the work that was done, recommendations can be made with respect to further work in this area using HPLC.

Without an extensive field investigation it was difficult to assess the significance of the measured biochemical oxygen demand of the Blindman River with respect to the oxygen budget of the Red Deer River. However, by evaluating the relative flow data of the Blindman River and similar tributaries upstream of Red Deer (Figure I-1) the

plausible bounds to the oxygen demand effects can be considered.

The four tributaries upstream of the City of Red Deer, shown in Figure I-1 comprise approximately 30% of the low winter flow of the Red Deer River at Red Deer, which is a greater contribution than during the summer months (Historical Stream Flow Summary - Alberta to 1973). The Blindman River, during this sampling period, was estimated to contribute as much as 6% of the total winter flow of the Red Deer River at Red Deer, with significantly lower contributions (3 to 0.1%) in the summer. The four tributaries upstream of Red Deer exhibit high organic loadings similar to those found in the Blindman River. Since significant biochemical oxygen demand can be demonstrated on natural organic runoff (humic and lignin-like substances in particular) at 20°C and at temperatures approaching winter, under ice, conditions, it is plausible that natural organic ruroff could cause the occurrence of a few mg/l of DO depletion during ice cover in reaches of the Red Deer River not subjected to man made pollution. It also appears that nitrogenous oxygen demand may play a significant role in the low temperature oxygen demand.

The findings of this investigation with respect to the nitrogen balance suggests that a field investigation may result in incorrect findings concerning nitrification if all factors are not taken into account. Two important factors which should be assessed are the possible occurrences of

denitrification and probably more important, in streams high in aquatic humus, losses of nitrogenous components through the binding capabilities of humic substances. Probably the best method of assessing the degree of nitrification would be, to perform electrolytic respirometer BOD tests on samples with and without a nitrifying inhibitor.

If the winter dissolved oxygen problem in the Red Deer River upstream of Red Deer is correctly attributed to natural organic loading then increased waste water treatment to reduce pollutant loading, would not be a feasible solution to the problem. Flow regulation appears to be the best long term practical solution to this problem, particularily if large increases in municipal and industrial growth is anticipated in the near future.

VII. RECOMMENDATIONS

1. Since an increase in the sensitivity of the electrolytic respirometer technique is desirable, it is recommended that a plexiglass sleeve be placed within the electrolytic cell, as demonstrated by Young and Baumann (1972).

2. Since changes in organic carbon levels are so small, the implementation of a more sensitive method of organic carbon analysis such as, photochemical oxidation (Poirier and Wood, 1978), is recommended.

3. It is recommended that sample concentration be achieved through freeze drying, to minimize chemical alterations. Another method of increasing the absolute mass of parameters within the reactor without modifying the samples' make-up, may be achieved by increasing the sample volume (10-20 litres), thus eliminating the sample concentration step. However, the behavior of an electrolytic respirometer has not yet been demonstrated for such large sample volumes.

4. A close monitoring of nitrite, nitrate and ammonia nitrogen is recommended throughout the course of a run, to give further information regarding the behavior or fate of these compounds in a sample high in humic substances. Spiking the samples with various concentrations of nitrite, nitrate and ammonia nitrogen and monitoring their behavior during a run should also be carried out. Several runs should also be conducted using a nitrifying inhibitor which does

not interfere with the chemical make-up of the sample. There is the possibility that some inhibitors may become bound to the aquatic humus and become ineffective, this should be investigated. It is recommended that the degree of nitrification be assessed by performing runs with and without a nitrifying inhibitor.

5. It is recommended that sample seeding be achieved through the use of a seed solution cultured from the sessile organisms found at the bottom of the river, to ensure that no new parameters are introduced.

6. Colour (based on a platinum-cobalt standard) is another parameter which may give further insight into the biological degradation of humus. Gjessing (1976) pointed out that there is a close correlation between colour and organic carbon. It must however be realized that colour of aquatic humus can be effected by UV radiation, pH, mineral adsorption and other factors along with biodegradation.

7. Since humic and lignin-like substances appear to be the most plausible causative factor for the observed oxygen demand, certain components of the compounds should be examined with respect to biodegradation. In the literature there has been some indication that a bacterial culture has been isolated which is capable of degrading phenolic compounds (many of which are components of humic substances). It is recommended that a sample containing aquatic humus be analyzed with respect to specific degradation components of humus, before and after each run.

The aquatic humus in the samples could be degraded using Alkaline-CuO oxidation (Hall and Lee, 1974) and specific compounds such as, resorcinol and catechol could be quantitatively measured using HPLC. This could be done on an HPLC by comparing the degraded sample with standard solutions of resorcinol and catechol, using an anion exchange column, an eluent gradient of about 0.02M to 6.0M of ammonium acetate over a particular time period and detecting at a wavelength of 254nm.

8. As a final recommendation, it is felt that an intensive study should be conducted on the benthic oxygen demend within this river system in order to assess its significance with respect to the winter dissolved oxygen deficit.

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APPENDIX

TABLE A1 Data from the electrolytic respirometer experiment at 20°C on concentrated river sample: July 13, 1978.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
8.3	204	17.0	50.0	-	-	-

20° Electrolytic Respirometer Experiment
(conc. factor* = 6.93, 6.93, 7.60 for R1, R2 and R3 respectively)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1	8.3	864	118.0	200.2	-	-	-
	2	8.2	836	119.6	206.8	-	-	-
	3	8.2	958	130.0	223.3	-	-	-
200	1	8.9	670	108.0	123.5	-	-	-
	2	8.8	650	112.0	126.0	-	-	-
	3	8.8	726	127.4	140.1	-	-	-

* with respect to liquid volume reduction

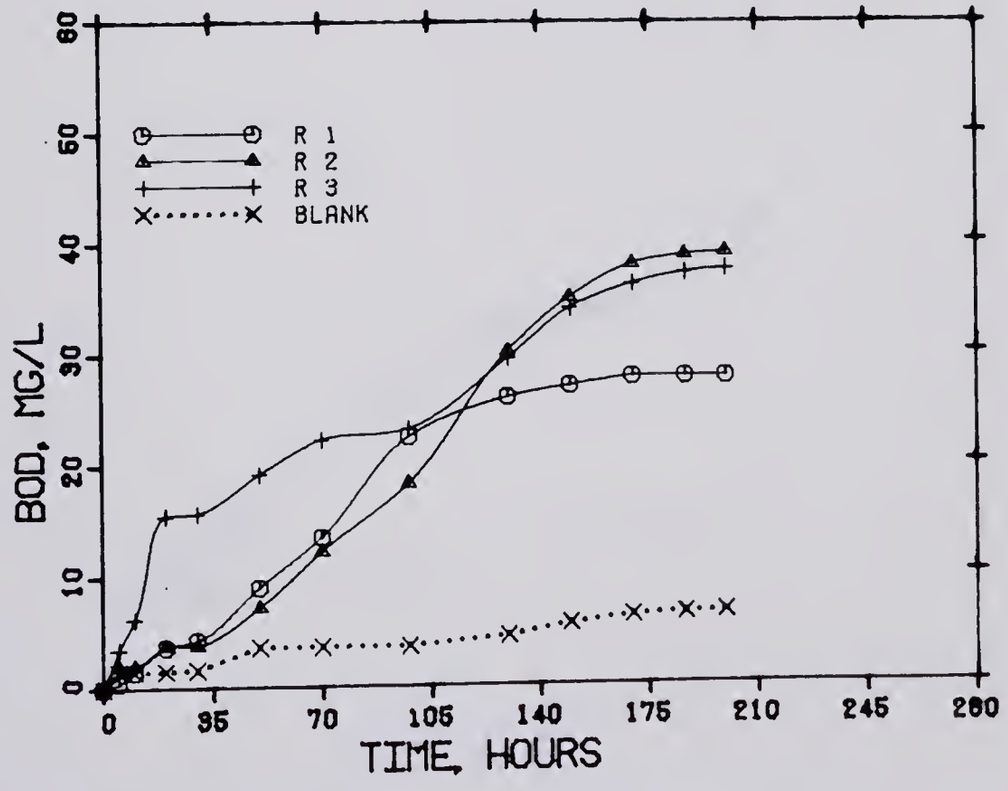

FIGURE A1. Oxygen demand curves of concentrated river sample at 20°C, July 13, 1978.

TABLE A2. Data from the electrolytic respirometer experiment at 20°C on concentrated river sample: July 24, 1978.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
8.4	202	17.0	44.0	-	-	-

20°C Electrolytic Respirometer Experiment
(conc. factor* = 7.60, 7.88, 7.80 for R1, R2, and R3 respectively)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1	8.3	986	110.0	211.5	-	-	-
	2	8.4	1120	122.0	244.0	-	-	-
	3	8.3	1130	124.0	265.0	-	-	-
210	1	-	-	-	-	-	-	-
	2	8.5	738	118.0	211.0	-	-	-
	3	8.5	856	118.0	216.0	-	-	-

* with respect to liquid volume reduction

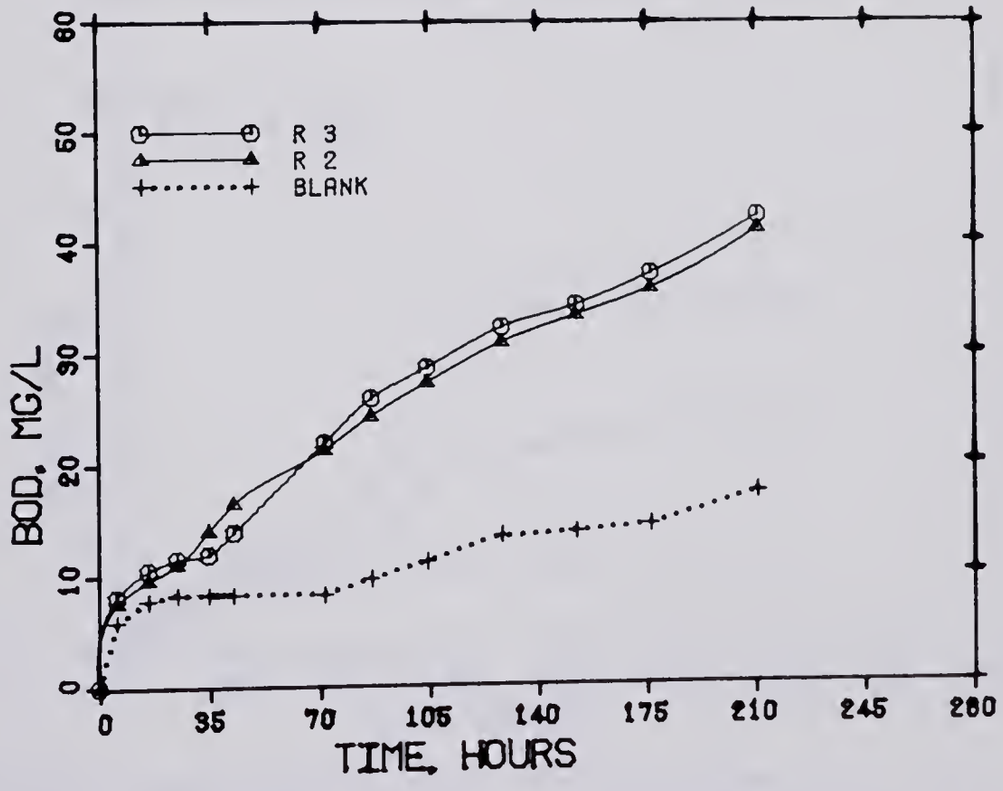

FIGURE A2. Oxygen demand curves of concentrated river sample at 20°C, July 24, 1978.

TABLE A3. Data from the electrolytic respirometer experiment at 20°C on concentrated river sample: Aug. 5, 1978.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l asC	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
8.3	205	16.0	42.0	-	-	-

20°C Electrolytic Respirometer Experiment
(conc. factor* = 7.78, 7.26, 7.82 for R1, R2, and R3 respectively)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1	8.2	1180	118.0	285.0	-	-	-
	2	8.1	1118	106.4	245.0	-	-	-
	3	8.2	1232	122.0	255.0	-	-	-
215	1	9.0	860	122.0	180.0	-	-	-
	2	8.9	812	118.0	168.0	-	-	-
	3	9.0	846	122.0	188.0	-	-	-

* with respect to liquid volume reduction

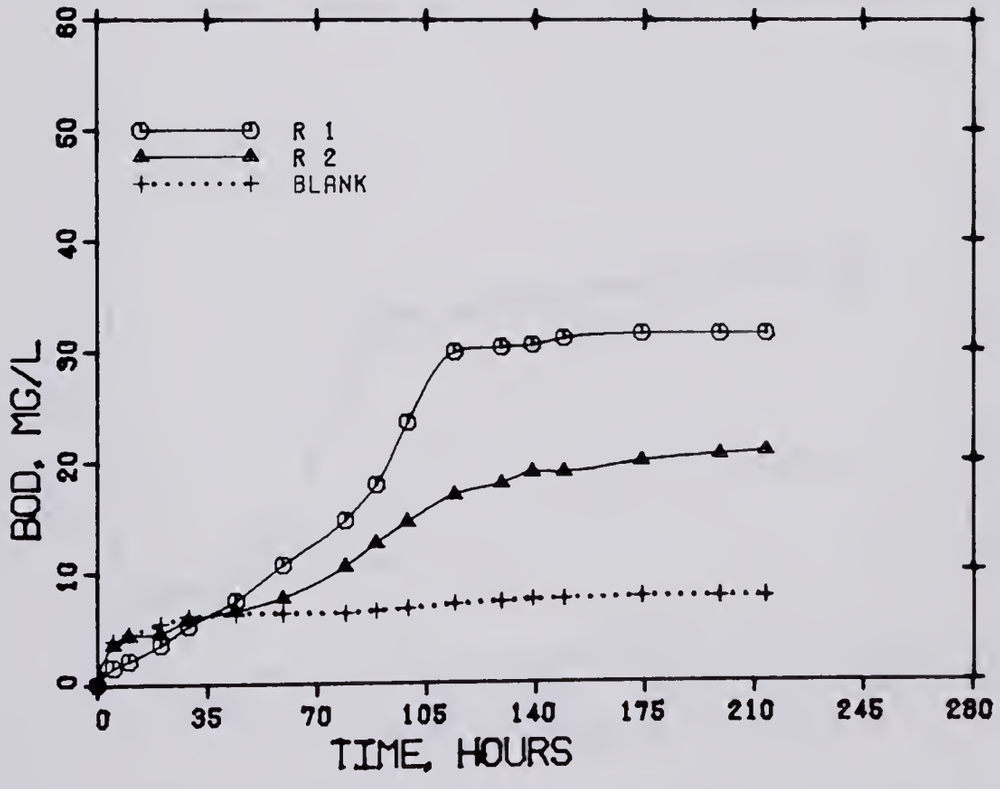

FIGURE A3. Oxygen demand curves of concentrated river sample at 20°C, Aug. 5, 1978.

TABLE A4. Data from the electrolytic respirometer experiment at 20°C on concentrated river sample: Aug. 23, 1978.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
8.2	180	14.0	45.8	-	-	-

20°C Electrolytic Respirometer Experiment
(conc. factor* = 8.46, 7.55, 7.67 for R1, R2, and R3 respectively)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1	8.0	1080	127.0	225.0	-	-	-
	2	8.0	1042	115.6	187.0	-	-	-
	3	8.1	1056	116.8	235.5	-	-	-
239.3	1	8.6	830	116.8	178.0	-	-	-
	2	8.6	778	97.2	161.5	-	-	-
	3	8.6	792	99.0	156.0	-	-	-

* with respect to liquid volume reduction

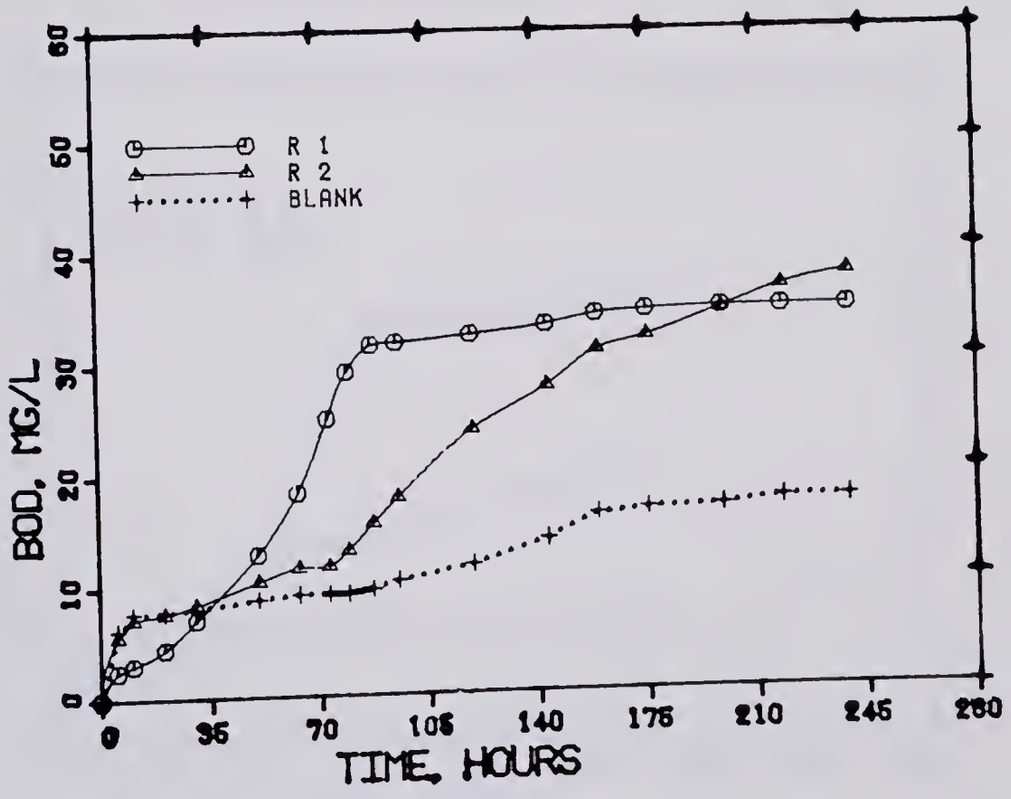

FIGURE A4. Oxygen demand curves of concentrated river sample at 20°C, Aug. 23, 1978.

TABLE A 5. Data from the electrolytic respirometer experiment at 20°C on concentrated river sample: Sept. 1, 1978.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
8.3	182	15.0	41.8	-	-	-

20°C Electrolytic Respirometer Experiment
(conc. factor* = 7.08, 7.79, 8.21 for R1, R2, and R3 respectively)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1	8.1	1032	105.2	215.0	-	-	-
	2	8.2	1082	110.2	237.5	-	-	-
	3	8.2	1222	120.3	268.0	-	-	-
200	1	8.7	854	99.0	180.0	-	-	-
	2	8.6	870	110.2	192.5	-	-	-
	3	8.6	954	116.0	187.5	-	-	-

* with respect to liquid volume reduction

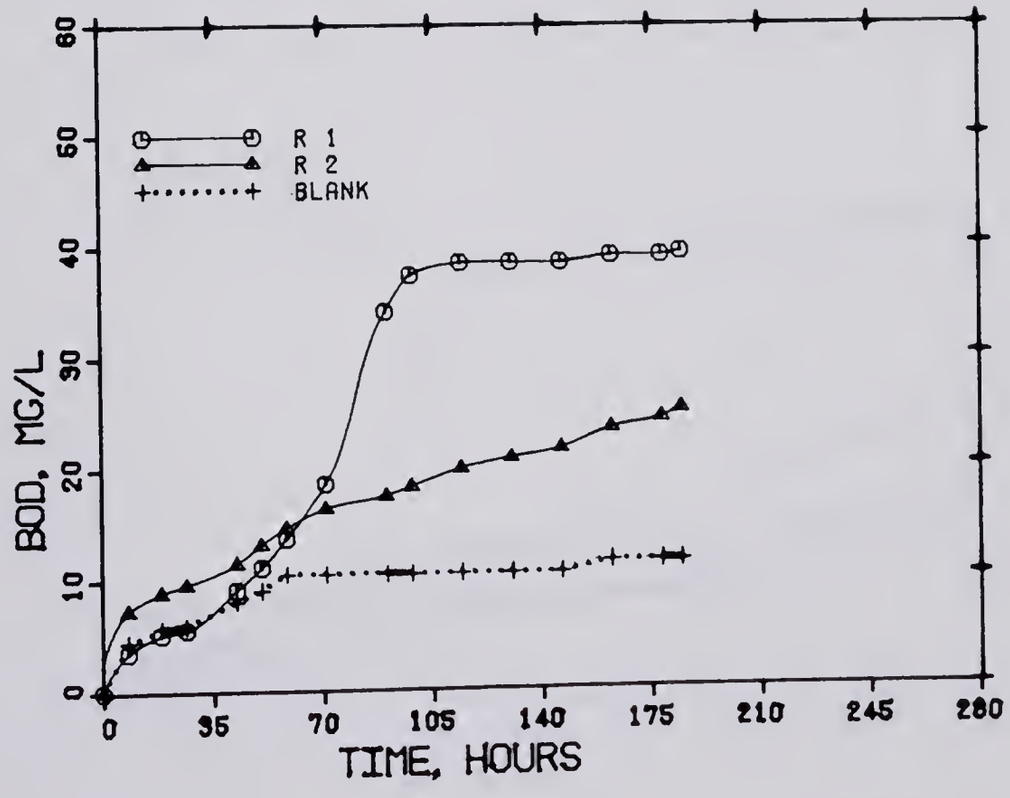

FIGURE A5. Oxygen demand curves of concentrated river sample at 20°C, Sept.1, 1978.

TABLE A6. Data from the electrolytic respirometer experiment at 20°C on concentrated river sample: Oct. 31, 1978.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
7.8	228	14.0	54.3	0.5	8.6	0.92

20°C Electrolytic Respirometer Experiment
(conc. factor*= 7.63 for R1, R2, and R3)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1,2,3	8.1	1128	110.0	194.5	0	28.3	5.01
247	1	8.7	598	107.8	156.0	0	3.9	3.73
	2	8.7	862	106.4	163.5	0	19.5	4.86
	3	8.9	860	106.0	140.5	0	1.3	5.06

* with respect to liquid volume reduction

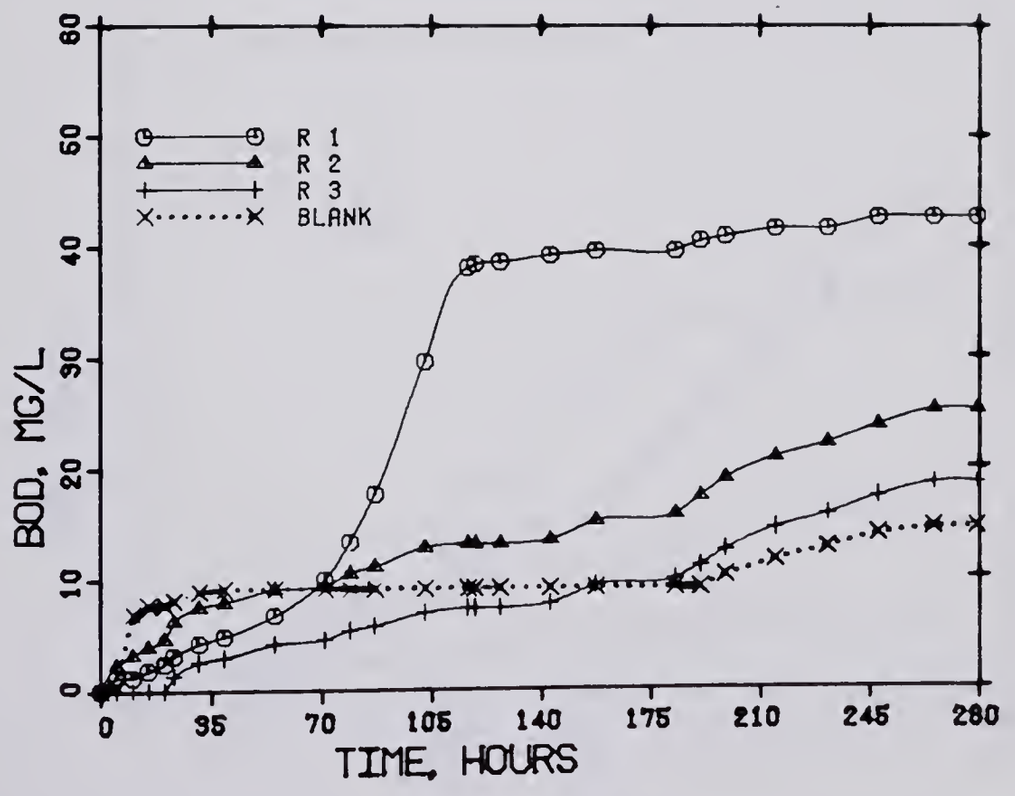

FIGURE A6. Oxygen demand curves of concentrated river sample at 20°C, Oct. 31, 1978.

TABLE A7. Data from the electrolytic respirometer experiment at 20°C on concentrated river sample: Nov. 10, 1978.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
7.8	225	12.8	39.2	2	1.9	0.66

20°C Electrolytic Respirometer Experiment
(conc. factor* = 7.65 for R1, R2, and R3)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1, 2, 3	8.2	1094	114.6	274.0	0	10.5	0.66
134.5	1	-	-	-	-	82	40.4	-
	2	-	-	-	-	82	42.6	-
	3	-	-	-	-	83	38.3	-
260	1	9.0	778	102.4	162.5	3.5	0	4.51
	2	9.0	790	104.2	163.0	3.0	10.3	4.59
	3	8.9	790	103.6	164.0	3.0	10.3	5.22

* with respect to liquid volume reduction

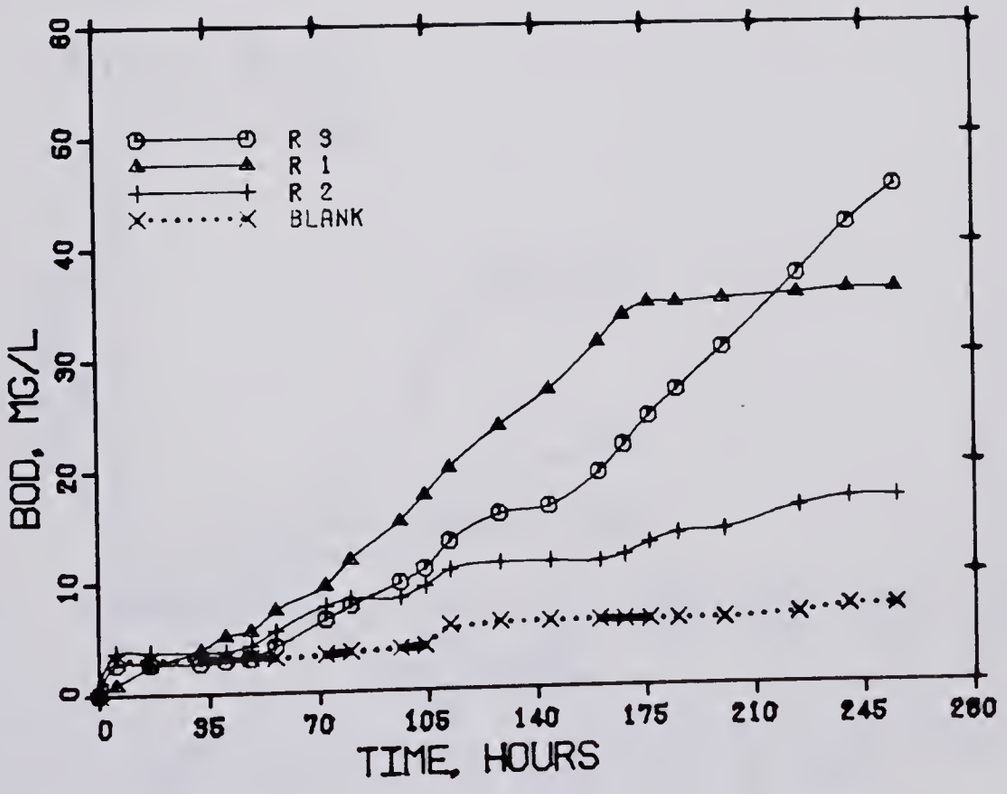

FIGURE A7. Oxygen demand curves of concentrated river sample at 20°C, Nov. 10, 1978.

TABLE A8. Data from the electrolytic respirometer experiment at 20°C on concentrated river sample with ATU added: Oct. 22, 1978.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
7.4	223	16.5	48.0	-	-	0.85

20°C Electrolytic Respirometer Experiment
(conc. factor* = 7.71 for R1, R2, and R3)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1,2,3	8.0	1110	115.4	184.0	5	113.3	4.56
175	1	8.8	1112	114.3	224.0	132	77.7	5.88
	2	8.8	1096	114.3	223.5	5	87.5	4.84
	3	8.8	1090	120.0	201.5	5	66.0	5.07

* with respect to liquid volume reduction

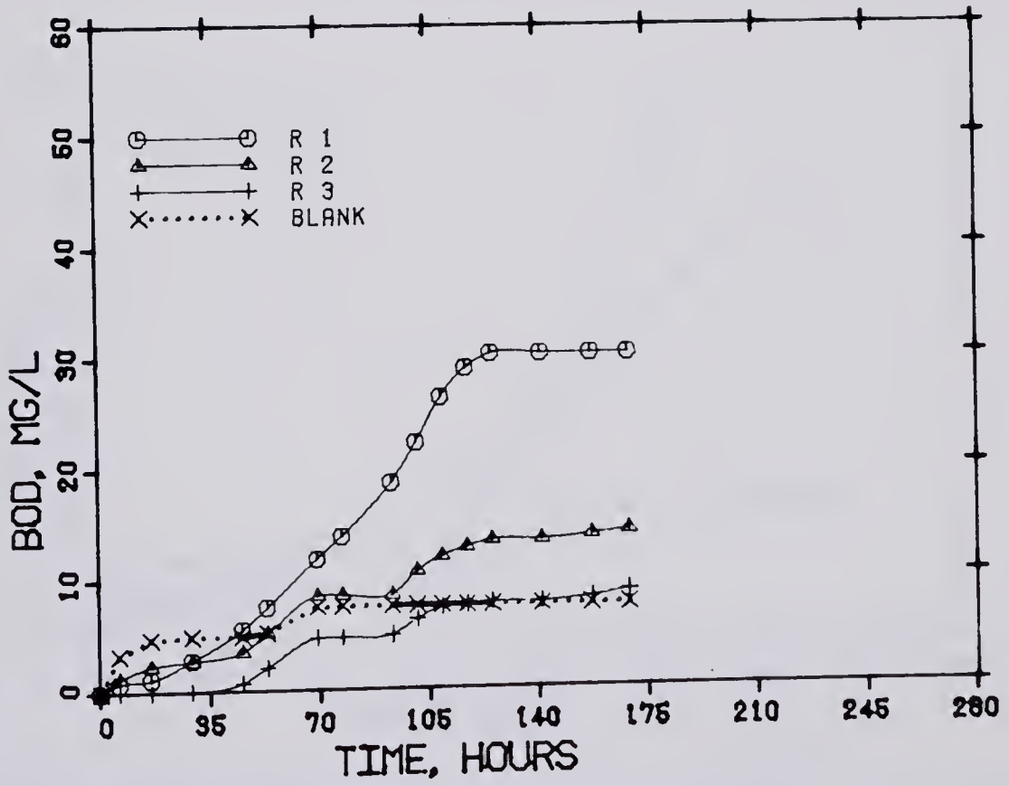

FIGURE A8. Oxygen demand curves of concentrated river sample at 20°C with 5 mg/l ATU added, Oct. 22, 1978.

TABLE A9. Data from the electrolytic respirometer experiment at 20°C on unconcentrated river sample: Oct. 4, 1978.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
7.7	179	17.5	36.5	-	-	-

20°C Electrolytic Respirometer Experiment
(conc. factor = 1.00 for R1, R2, and R3)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1,2,3	7.7	179	17.5	36.5	-	-	-
238.8	1	8.7	180	17.8	29.4	-	-	-
	2	8.6	180	17.2	27.8	-	-	-
	3	8.6	180	16.0	34.6	-	-	-

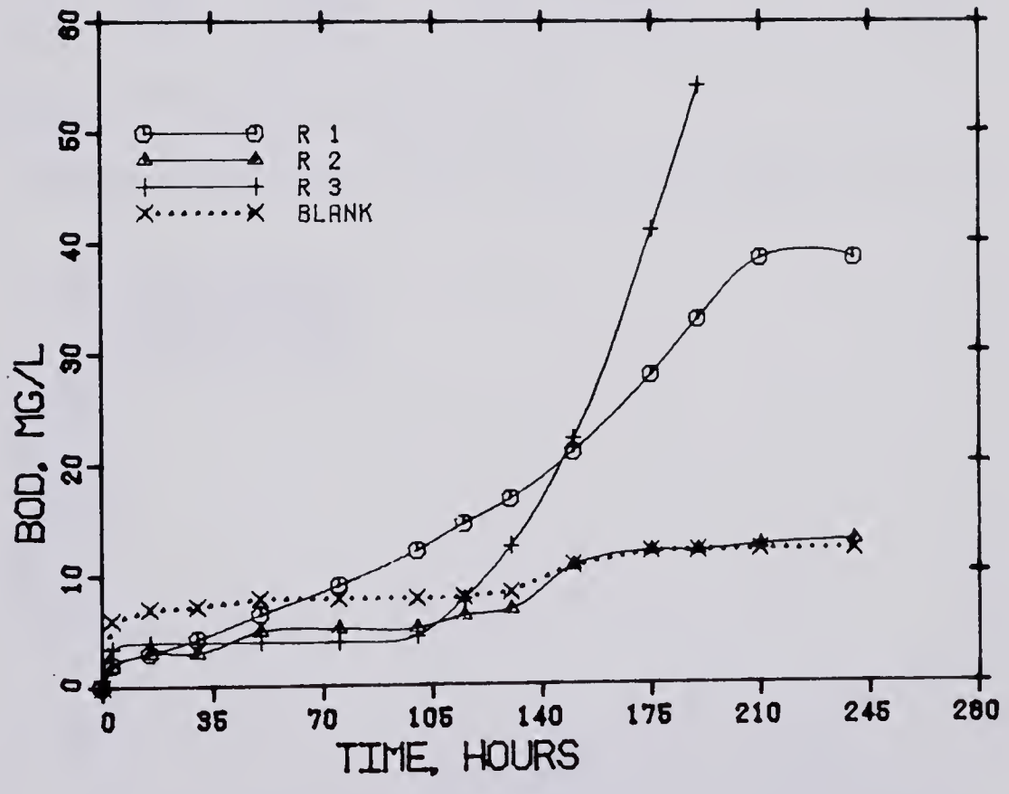

FIGURE A9. Oxygen demand curves of unconcentrated river sample at 20°C, Oct. 4, 1978.

TABLE A10. Data from the electrolytic respirometer experiment at 4°C on concentrated river sample: Nov. 23, 1978.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
7.5	299	17.4	72.0	2.2	24.9	0.95

4°C Electrolytic Respirometer Experiment
(conc. factor* = 7.36 for R1, R2, and R3)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1,2,3	8.1	1274	117.2	310.5	0	1	4.95
145.7	1	-	-	-	-	9	73.0	-
	2	-	-	-	-	4	89.0	-
	3	-	-	-	-	6	63.0	-
263.8	1	9.0	1014	113.6	222.0	3	77.6	5.16
	2	8.9	1044	115.0	228.0	1	87.3	5.30
	3	9.1	1028	116.6	217.5	3	71.0	5.20

* with respect to liquid volume reduction

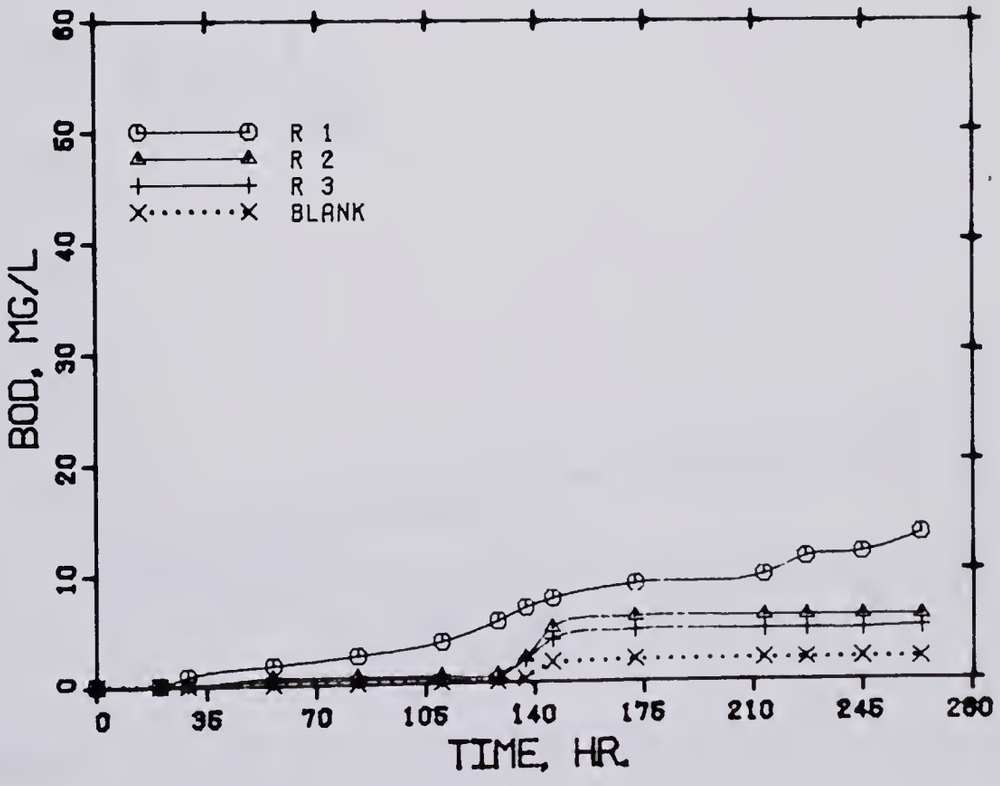

FIGURE A10. Oxygen demand curves of concentrated river sample at 4°C, Nov. 23, 1978.

TABLE A11. Data from the electrolytic respirometer experiment at 4°C on concentrated river sample: Dec. 14, 1978.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
7.7	318	15.0	78.5	2	74.0	1.0

4°C Electrolytic Respirometer Experiment
(conc. factor* = 7.53 for R1, R2 and R3)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1,2,3	8.3	1424	112.0	321.0	0	2.1	5.36
261.5	1	9.0	1140	111.0	261.0	0	418.4	4.78
	2	8.9	1048	106.1	248.9	1	417.4	4.56
	3	9.0	1124	114.0	256.2	0	428.6	4.92

* with respect to liquid volume reduction

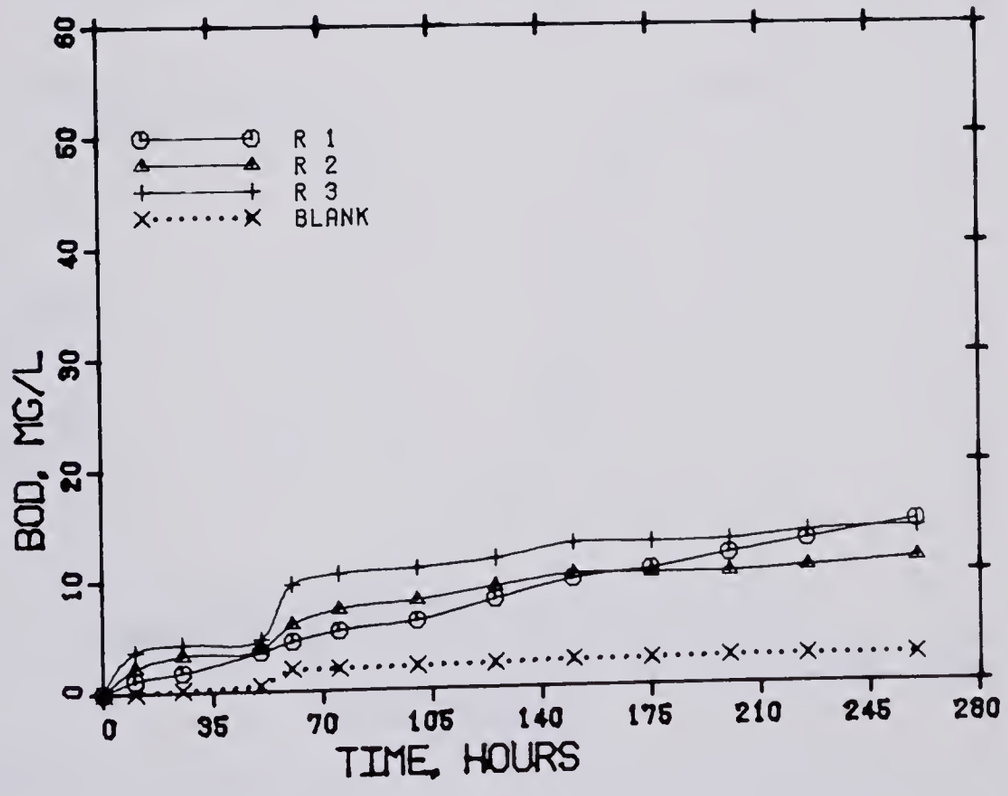

FIGURE A11. Oxygen demand curves of concentrated river sample at 4°C, Dec. 14, 1978.

TABLE A12. Data from the electrolytic respirometer experiment at 4°C on unconcentrated river sample: Jan. 4, 1979.

River Sample - Original Concentration

pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN ug/l as N
7.3	389	15.0	90.3	2	142.0	0.84

4°C Electrolytic Respirometer Experiment
(conc. factor = 1.00 for R1,R2 and R3)

Time hours	Reactor	pH	Tot. Alk. mg/l as $CaCO_3$	TOC mg/l as C	IC mg/l as C	NO_2^- µg/l as N	NO_3^- µg/l as N	TKN mg/l as N
0	1,2,3	7.3	389	15.0	90.3	2	142.0	0.84
225	1	8.3	230	14.8	56.0	23	132.7	0.64
	2	8.1	250	14.7	60.8	0	146.9	0.73
	3	8.2	226	14.4	61.6	0	110.6	0.74

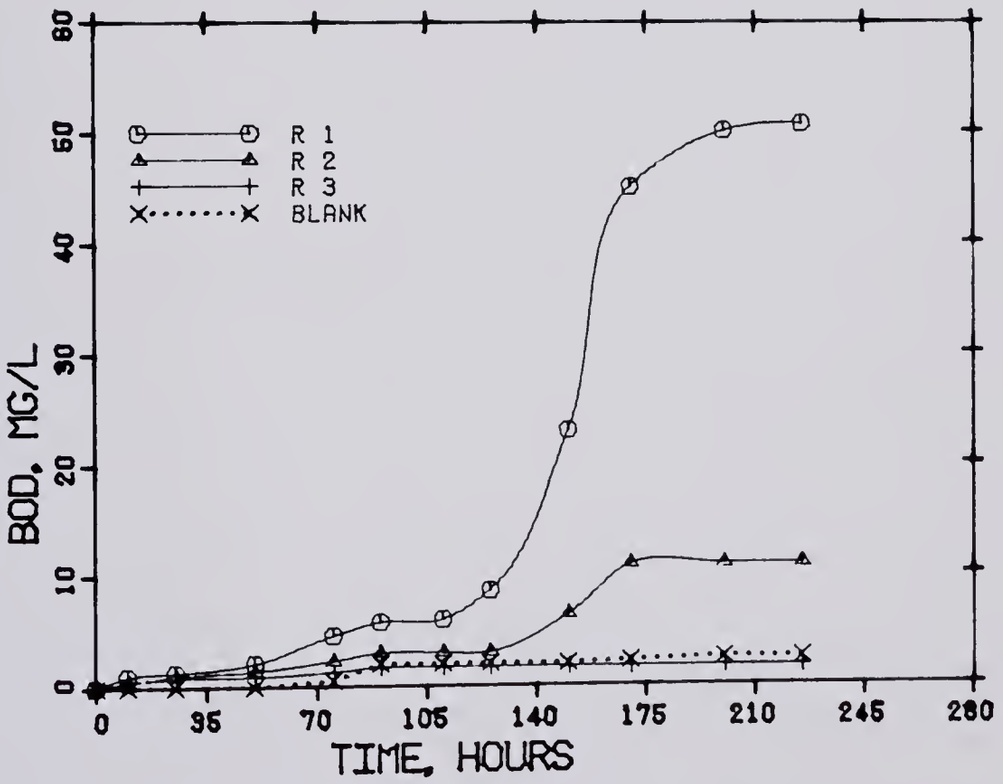

FIGURE A12. Oxygen demand curves of unconcentrated river sample at 4°C, Jan. 4, 1979.

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